

THE IMPORTANCE OF LUMINAL CONTENTS IN INTESTINAL ADAPTATION
AND EXPERIMENTAL COLORECTAL CARCINOGENESIS

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PREFACE

The experimental work for this thesis was carried out in the University of Bristol Department of Surgery, Bristol Royal Infirmary where I held the post of Research Fellow and Honorary Senior Registrar from February 1982 until September 1983. The research was generously supported by the Cancer Research Campaign, to whom I am deeply indebted, and inspired and supervised by Professor R C N Williamson, Professor of Surgery at the University of Bristol. I am sincerely grateful to him for his advice, based on wide knowledge and experience of the fields of research covered in this work, and for his constant encouragement and constructive criticism of the research as it developed.

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ABSTRACT

The incidence and geographical distribution of colorectal cancer in man are discussed and the history and development of various experimental animal models of the disease described. Epidemiological and experimental evidence relating to the many possible aetiological factors in large bowel cancer are reviewed with particular reference to the potential role of bile acids as endogenous cocarcinogens. Current knowledge of the phenomenon of intestinal adaptation to various stimuli and its possible link with carcinogenesis is discussed.

A series of six experiments employing a well-established animal model of colorectal cancer are described. These were designed to examine the importance of luminal factors in adaptation and carcinogenesis and to investigate further the relationship between the proliferative state of the bowel mucosa and its susceptibility to neoplastic change. Several broad conclusions were reached: (1) Surgical shortening of the gut produces mucosal hyperplasia and enhanced carcinogenesis in residual functioning bowel. (2) By contrast, mucosal proliferation and carcinogenesis are depressed in bowel deprived of luminal contents. (3) Bile acids are tropic and strongly cocarcinogenic in colon-in-continuity but not in defunctioned colon. (4) Bacteria play an important part in modulating the cocarcinogenic potential of bile acids but have no influence on bile acid-induced mucosal hyperplasia.

The results of the laboratory studies are discussed in the light of current knowledge of experimental and human colorectal carcinogenesis. They support the thesis that luminal contents in general and bile acids in particular are crucial factors governing both the adaptive response of the bowel to various stimuli and its susceptibility to carcinogenesis.

CHAPTER 1
INTRODUCTION

Cancer of the large intestine kills some 17,000 patients every year in England and Wales (Registrar-General, 1978) and it is equally prevalent in most other developed countries. Only a third of patients survive 5 years after presentation (Goligher, 1984), a mortality rate which has remained essentially unchanged in Britain since the Second World War. At present, radical surgery still provides the only realistic hope of cure, yet half of those patients undergoing "curative" resection develop recurrent cancer within 5 years. The relatively occult nature of early colorectal cancer frustrates attempts to increase survival by improvements in clinical management and surgical techniques. Almost all Dukes stage A lesions are cured by resection but many patients remain symptom-free until the disease is more advanced and their chance of surviving is therefore markedly reduced (Lockhart-Mummery et al., 1976; Gill and Morris, 1978). Improved methods of early diagnosis and the development of simple reliable screening techniques could, therefore, reduce the mortality rate appreciably but financial and logistic constraints probably prohibit effective screening of the vast population potentially at risk.

Another approach to the problem of colorectal cancer is the study of its aetiology with the ultimate objective of the identification of its cause and the introduction of appropriate preventive measures. In the long term it seems likely that sensible prophylaxis, rather than early detection or improved treatment, will have the major impact on deaths from cancer of the large intestine. Current knowledge of the aetiology of intestinal cancer is reviewed in this thesis, with particular reference to the importance of the mucosal proliferative state and the luminal environment. The roles of intestinal adaptive hyperplasia and the composition of bowel contents are further explored in a series of experiments employing a well-established animal model.

The incidence and geographical distribution of large bowel cancer are

described in Chapter 2. It is a disease which primarily affects Western industrialised nations (Burkitt, 1971). Migrant groups from countries where the incidence is low tend to become as susceptible as the indigenous population of their adopted country within one or two generations (Haenszel and Correa, 1971). This epidemiological evidence suggests that the risk of colorectal cancer is determined by the environment to a much greater degree than by heredity.

Since experiments in man would be subject to major ethical and practical constraints, aetiological studies of colorectal cancer rely heavily on experiments carried out on laboratory animals in which spontaneous intestinal cancer is exceedingly rare. In Chapter 3, the numerous chemical compounds found to induce intestinal neoplasms in various species are reviewed. The chemistry, pathology and advantages of the most successful model (cycasin-group derivatives administered to rodents) are described.

The major proposed aetiological factors in human large bowel cancer are discussed in Chapter 4. Diet appears to be the main influence. Western populations differ from those in the Third World in that they consume more calories and less fibre. Various dietary constituents, fat, cholesterol, protein and alcohol are consumed in greater quantities by susceptible populations. Unlikely to be directly carcinogenic to colorectal mucosa, these substances may exert their effect by influencing the amounts of endogenous substrates such as bile acids and neutral sterols reaching the colon and by governing the population of faecal bacteria which could convert them to active carcinogens (Heaton, 1977). The majority, if not all, colorectal carcinomas may arise in pre-existing adenomas. The increasingly compelling evidence supporting the case for the "adenoma-carcinoma sequence" is presented in this chapter. Chronic inflammation may be implicated as the risk of carcinoma is increased

in patients with longstanding ulcerative proctocolitis (Lennard-Jones et al, 1977) and probably also in those with Crohn's colitis (Weedon et al, 1973). Injury, whether due to pelvic irradiation or trauma at stomas and suture lines could also be an important promoter of intestinal cancer (Martins et al, 1980; Pozhariski, 1975).

The gut mucosa is the site of continuous cell proliferation with turnover times of 2-8 days in rodents and man (Williamson, 1978). Normal intestinal cell kinetics and preneoplastic and neoplastic changes are described in Chapter 5. The normally rapid rate of mucosal cell renewal can be intensified by a number of stimuli, the strongest of which is surgical resection or bypass (Williamson, 1978). The phenomenon of adaptive hyperplasia in residual bowel following loss of a functioning segment and in response to other non-surgical stimuli such as lactation, diabetes, hyperphagia and cold acclimatisation, has been extensively studied in the small bowel and current concepts are discussed in Chapter 6. On the other hand present knowledge of colonic adaptation is comparatively sparse and is reviewed in Chapter 7.

Cell proliferation is an essential prerequisite for carcinogenesis (Cayama et al, 1978). In man, promoting factors such as ulcerative proctocolitis and irradiation stimulate cell proliferation in colonic crypts. The hypothesis that hyperplasia promotes neoplasia is supported by animal experiments showing that surgical procedures which induce intestinal hyperplasia (adaptation) also enhance intestinal carcinogenesis (Williamson, 1982a). This proposed link between adaptation and cancer is discussed in Chapter 7. Luminal factors seem to have a key role in the control of adaptive hyperplasia (Williamson, 1978) and may therefore also influence carcinogenesis. Bile acids, in particular, are strongly implicated as major colorectal cocarcinogens (Reddy, 1981; Thompson, 1982). The epidemiological and experimental evidence

supporting this contention and the complex relationship between bile acids and faecal bacteria are reviewed in Chapter 8.

In Chapter 10, a series of six experiments designed to investigate the relationship between luminal factors, adaptive hyperplasia and carcinogenesis are described.

In conclusion, Chapter 11 will discuss the results of the laboratory studies in the light of current knowledge of experimental and human colorectal carcinogenesis.

CHAPTER 2

INCIDENCE AND DISTRIBUTION OF HUMAN COLORECTAL CARCINOMA

Overall Prevalence

Carcinoma of the large bowel is the second commonest cause of death from cancer in males in the Western world after lung cancer (Burkitt, 1971). It is responsible for about 17,000 deaths per year in England and Wales. In the United States of America it is second only in incidence to skin cancer: 100,000 new cases are seen every year and the annual number of deaths approaches 50,000 (Pratt et al, 1977). Gilbertsen in 1974 stated that 4.5 million Americans alive at that time would eventually die of this disease, and MacLennan in 1978 calculated that men in Connecticut surviving to the age of 74 had a 1 in 30 chance of developing colon cancer. Since World War II the incidence of colon cancer in the industrialised countries has remained stable or shown a slight increase, whereas that of rectal cancer has decreased (Wynder, 1975).

In 1964 colorectal cancer accounted for 14% of all deaths from cancer in America, more than any other cancer except bronchial carcinoma (Wynder and Shigematsu, 1967). By 1977 it had become the leading cause of cancer mortality, responsible for 25% of all deaths from malignant disease (Copeland, 1977; Pratt et al, 1977). In Britain the overall mortality rate has not changed since 1945 (Goligher, 1984). The slight increase in incidence has been balanced by improved survival.

Geographical distribution

The geographical distribution of a disease may be difficult to identify owing to poor data collection in some countries coupled with inadequate pathological confirmation of clinical diagnosis. These problems, however, do not obscure the picture for colorectal cancer. It is predominantly a disease of the Western world (USA, Western Europe and Australasia), where it is 10 times

more common than in Africa or Asia (Doll, 1969; Burkitt 1971). The highest incidences are reported in the USA, New Zealand, Scotland and Canada, while the disease is comparatively rare in the Third World (Berg and Howell, 1974; Copeland, 1977; Correa, 1978; Doll, 1980). Incidences per 100,000 (standardised for males aged 35-64) range from 51.8 in Connecticut, USA to 3.5 in Kampala, Uganda. Within the United Kingdom, Scotland has the highest incidence (51.5 compared with 37.7 in England and Wales) (Burkitt, 1971).

No other disease is so closely linked with industrialisation and economic development (Higginson, 1966). There are exceptions, however. The low incidence in Japan belies its national affluence, while there is a high incidence of large bowel cancer in Argentina which remains a relatively poor country (Stewart, 1971; Berg and Howell, 1974; Correa, 1978).

Variations in incidence within a country are much less marked than those between countries. The socioeconomic gradient does not appear to be important (Correa, 1978). Rural populations in the UK, USA and Scandinavia may develop fewer colorectal cancers than their urban counterparts (Haensel et al, 1973). There are minor variations between different religious sects in the United States where Mormon and Seventh Day Adventists have a lower incidence and Jews have a higher incidence than the rest of the population (Wynder and Shigematsu, 1967; Philips, 1975). In Israel the disease is about three times more common in Jews of European extraction than in Jews of African and Asian extraction (Horovitz and Huber, 1980).

The predilection of colorectal cancer for developed "westernised" countries implies a strong environmental factor in its aetiology. Indeed epidemiological studies on migrant populations confirm that the environment is more important in this respect than racial or genetic factors. Japanese immigrants to Hawaii and California have progressively developed

susceptability to intestinal cancer comparable with that of the indigenous population and much higher than that in their country of origin (Wynder and Shigematsu, 1967; Stemmermann, 1970; Haensel and Correa, 1971). Presumably colorectal cancer was rare in Africans first imported as slaves into the United States, but by 1947 the incidence in American negroes was half of that in caucasians (UB Public Health Monograph, 1956). By 1969 the disease was equally prevalent in both populations (Doll, 1969). This trend is repeated in other ethnic groups (Puerto Ricans, Poles, Norwegians and Chinese), who have a much higher incidence in the USA than in their countries of origin.

Sex/Age Incidence

Cancer of the large bowel occurs with roughly equal frequency in men and women (Berg and Howell, 1974; MacLennan, 1978). In most countries the male/female ratio of colon cancer approaches unity, with a slightly higher incidence in females particularly in the first six decades (Wynder, 1975; Copeland, 1977). Exceptions to this rule include Rhodesia and Hawaii where the disease is more common in men of all ages (Stewart, 1971). Carcinoma of the rectum is slightly more common in males than females. These admittedly slight differences in sex incidence between colonic and rectal cancer lend support to the theory of two different diseases with different aetiologies (Wynder and Shigematsu, 1967; Berg and Howell, 1974; Copeland, 1977; MacLennan, 1977). In populations which migrate from a region of low risk to a high-risk country, males are the first to show an increased incidence but females eventually catch up (Correa, 1978).

Colorectal cancer is predominantly a disease of the elderly and its incidence increases progressively with age. This pattern is consistent with the theory of cumulative exposure to an intestinal carcinogen. In the Western

world the incidence peaks in the seventh and eighth decades and less than 5% of cases occur in those under 35 (Pratt et al 1977; Dajani et al, 1980; Goligher, 1984; Horovitz and Huber, 1980; Umpleby and Williamson, 1984). In regions and populations with a low prevalence it tends to present earlier with peaks in the fifth and sixth decades (Dajani et al, 1980; Elliot and Louw, 1979). In one 15 year survey in Kinshasa, Zaire, large bowel cancer accounted for only 5% of all solid tumours, and 43% of these were found in patients under 35 (Ngala Kenda, 1976). In Israel, Jews of Afro-Asian descent who have a much lower overall incidence of the disease than Jews of American-European descent, have significantly higher incidence in the under 50 age group (Horovitz and Huber, 1980).

This cancer is extremely rare in those under 30 and only 1-2% of all cases are found in this age group (Goligher, 1984). Colorectal cancer occurs only sporadically in children and adolescents; less than 300 cases have been recorded in the literature (Pratt et al, 1977). In this group the disease has several different features from that normally seen. Pain is a common presenting symptom, the diagnosis is delayed and the prognosis is dismal (Symonds and Vickery, 1976; Pratt et al, 1977; Elliot and Louw, 1979; Dajani et al, 1980). Associated with aggressive invasiveness and local recurrence, mucinous cancers occur more frequently in the young than in large bowel cancer patients overall (Umpleby and Williamson, 1984). Mucinous tumours are also more common in low incidence populations, occurring in 16% of Cape coloureds (Elliot and Louw, 1979), 26% of Zairois (Ngala Kenda, 1976), and 31% of Jordanians (Dajani et al, 1980).

Anatomical Distribution

For many years the accepted teaching was that approximately one-half

of all large bowel cancers could be detected by digital examination and two-thirds by rigid sigmoidoscopy (Smiddy and Goligher, 1957). Nowadays these proportions are probably nearer one-quarter and one-half respectively (Morgenstern and Lee, 1978). Several large retrospective surveys have shown a marked left to right shift in tumour distribution (Cutler, 1969; Morgenstern and Lee, 1978; Rhodes et al, 1977). Cady et al (1974), reviewing 5,807 cases presenting over a 40 year period found that the percentage of right colonic tumours rose from 7 to 22 while that of sigmoid, rectosigmoid and rectal tumours fell from 80 to 62. As both tumour size and incidence of lymph node metastases had also fallen in the latter group they suggested that this trend was due to improved detection of tumours and better follow-up and treatment of adenomatous polyps arising in this region. Despite the advent of the colonoscope, improvement in detection of the relatively occult proximal colonic tumours may be much more difficult to achieve without massive screening programmes because of their generally later clinical presentation. Mammaza and Gordon (1982) reviewed 1,058 cases of colorectal carcinomas presenting over 24 years and found that the proportion of both right-sided and sigmoid tumours had doubled while the proportion of transverse and descending colonic lesions had remained stable. Moreover, there was a very marked decrease of rectal tumours from 53 to 2% which, the authors assert, did not reflect changes in anatomical definition of the sigmoid, rectosigmoid and rectum.

It appears that the proportion of sigmoid cancers remains high in high incidence populations despite the increase in caecal and ascending colonic tumours. In low incidence populations, however, there is a more even distribution throughout the large bowel (Philips, 1975; Wynder, 1975; Pratt et al, 1977).

The incidence of rectal cancer tends to correlate with that of colonic cancer except in Japan, which has a relatively high rectal cancer rate despite a low incidence colonic cancer (Haensel and Correa, 1971; Berg and Howell, 1974; Wynder, 1975). This correlation also breaks down in other low incidence populations (Jordan and Zaire), suggesting that the colon is more sensitive than the rectum to environmental influences (Ngala Kenda, 1976; Dajani et al, 1980).

Within the rectum, tumours are evenly distributed between upper, middle and lower thirds (Goligher, 1984). Squamous carcinoma of the anus should be regarded as a completely separate disease.

Multiplicity

Cases of multiple colorectal carcinoma were first reported in the late nineteenth century (Czerny, 1880; Fenger, 1888). These were regarded as mere curiosities until Bargen and Rankin in 1930 drew attention to the distinct possibility of a second primary tumour occurring either at the same time as the index lesion or subsequently. Slaughter (1944) identified the large bowel as comparable to the skin and bladder as a site for multiple primary malignancies. This is perhaps to be expected of an organ 150 cm long with an epithelial surface of at least 1,000 cm² constantly exposed to much the same environmental factors throughout its length (Bussey, 1978).

The incidence of multiple large bowel cancer quoted in large series ranges from 3.1 - 5.5% (Moertel et al, 1958; Diamante and Bacon, 1966; Schottenfeld et al, 1969; Bussey, 1978; Enker and Dragacevic, 1978; Lee et al, 1982). Of these, about two-thirds are synchronous and one-third metachronous. Heald and Bussey (1975) have found the incidence of synchronous cancer to be 3.5%, and have estimated a 3.5% cumulative risk of

subsequently developing a metachronous tumour. Schottenfeld et al (1969) express the cumulative risk as 3.5/year/1,000 patients at risk. This is three times the expected risk in a normal age-matched population. Figures for the incidence of metachronous cancer, rely on thoroughness of follow-up and may be artificially inflated by synchronous cancers missed at the original operation (Heald and Lockhart-Mummery, 1972). However this problem is offset by the concept that many patients who die prematurely from other causes would have developed metachronous cancer had they survived.

When two tumours are found at the initial operation, the cumulative risk of a third (metachronous cancer) is 8% (Heald and Bussey, 1975), and patients surviving the resection of a second (metachronous) tumour have an 11% chance of developing a third lesion (Agrez et al, 1982). The presence of one or more adenomas in the resected specimen doubles the risk of a subsequent metachronous lesion. Hence the incidence of multiple tumours in familial polyposis is 48% (synchronous 41%, metachronous 7%) (Bussey, 1978).

The mean time interval between first and second lesions is variously quoted as 5.5 - 13.5 years with a range of 1 - 35 (Heald and Lockhart-Mummery, 1972; Morson, 1974; Agrez et al, 1982). The initial tumours in patients developing metachronous tumours tend to be less advanced and more favourable histologically than colorectal cancers overall (Bussey et al, 1967; Agrez et al, 1982). Clearly these patients are more likely to survive to develop their second cancers. The clear multifocal potential of large bowel cancer has strong implications for the initial investigation and eventual follow-up of all patients presenting with this disease.

Large bowel cancer is also associated with a 40% greater than normal chance of developing a second malignancy elsewhere, notably in the skin, breasts and genitourinary tract (Schottenfeld et al, 1969). The incidence of a

second extra-intestinal primary is quoted as 3.0 - 4.5% (Diamante and Bacon, 1966; Adelstein et al, 1979; Lee et al, 1982). The chance of developing a second malignancy either within the bowel or elsewhere appears to be greatest if the index cancer is situated in the caecum (Schottenfeld et al, 1969).

CHAPTER 3
EXPERIMENTAL COLORECTAL CARCINOGENESIS

Spontaneous Colorectal Cancer

Spontaneous colorectal cancer is an exceedingly rare occurrence in domestic and wild mammals with the exceptions of the dog and the cat (Roberts, 1960; Rowlatt, 1967; Lingeman and Garner, 1972; Pozharriski, 1973). In animals bred for commercial use the incidence may well be underestimated as unlike dogs and cats they are seldom allowed to live their normal lifespan. Not one colorectal neoplasm was found in 100,000 trapped wild rats screened for plague (McCoy, 1909). Tumours of other organs are much more common in rodents than large bowel lesions. One caecal carcinoma and 520 other neoplasms were detected in 32,000 laboratory rats submitted to autopsy (Curtis et al, 1931).

There have been 11 reported cases of spontaneous colorectal cancer in rats (Williamson et al, 1982). The disease is equally rare in mice (Rowlatt, 1967; Pozharriski, 1973). Self-limiting outbreaks of proximal colon cancer among inbred rats in two laboratories were probably caused by epizootics (Heslop, 1969; Miwa et al, 1976), which may also be responsible for occasional outbreaks of intestinal adenomatosis in pigs (Rowland et al, 1973; Roberts et al, 1980). Syrian hamsters may be slightly more susceptible to spontaneous colorectal cancer than other rodents (Herrold, 1969; Lingeman and Garner, 1972), but there appears to be marked clustering in certain colonies (Homburger et al, 1972).

Cancers can arise at colostomies or colonic anastomoses in some strains of rat (Williamson et al, 1982; Winkler et al, 1982), but these are probably sites of chronic irritation or inflammation and the cancers are not truly "spontaneous".

The rarity of the disease in animals has hindered the development of a satisfactory animal model of spontaneous colorectal cancer and experimental tumours have to be produced by artificial means.

Experimental tumours in other systems can be produced by viruses, but although particles characteristic of RNA tumour viruses have been described in some human large bowel cancers (Cuatrecasas et al, 1974), no microbial agent has yet been shown to produce intestinal cancer in man. Therefore apart from irradiation (discussed later in this review) most laboratory models rely on chemical carcinogens.

Chemical Carcinogens

A large number of chemicals have now been shown to have a carcinogenic action on rodent intestinal mucosa.

The first group of compounds identified as intestinal carcinogens were the polycyclic hydrocarbons. Methylcholanthrene and dibenzanthracene produced small bowel carcinomas and occasional large bowel tumours when administered orally in an oil emulsion to rats (Lorenz and Stewart, 1940). Methylcholanthrene was also effective when given directly into the stomach of hamsters (Della Porta, 1961), and topical administration by means of an impregnated string induced a high incidence of caecal carcinomas (Horava and Von Haam, 1958). The usefulness of this group as intestinal carcinogens is diminished by their toxicity and tendency to produce tumours in other organs. Similar problems have prevented routine use of other carcinogens such as benzidine and aflatoxin B1 (Spitz et al, 1950; Newberne and Rogers, 1973; Ward et al, 1975).

The second major group of intestinal carcinogens is the aromatic amines. Bielschowsky (1944, 1946) demonstrated the production of small bowel neoplasms in rats given 2-acetyl-aminofluorene orally, with a much lower incidence of colonic tumours. Different strains had markedly varying susceptibilities to this substance, which commonly produced tumours in

other organs. The first selective intestinal carcinogens were 4-aminobiphenyl and its derivatives particularly 3:2'-dimethyl-4-aminobiphenyl (DMAB) (Walpole et al, 1952). Administered parenterally, DMAB is secreted in the bile and acts directly on the bowel mucosa. It is therefore not carcinogenic to bowel segments sequestered from the faecal stream (Cleveland et al, 1967; Navarrete and Spjut, 1967).

N-nitroso compounds make up the third group of intestinal carcinogens. These are mostly nitrosamides which do not require metabolic activation (Lijinsky, 1977). The most useful is N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), which when given to rats by rectal infusion produces a high incidence of large bowel adenomas and carcinomas and occasional small bowel tumours (Pozharriski et al, 1979). Given by mouth it produces gastric and duodenal cancers. Alkyl nitrosoureas and urethanes can induce intestinal tumours after only a single administration (Schoental and Bensted, 1968; Leaver et al, 1969; Ward et al, 1977).

Animals fed bracken fern (*Pteridium aquilinum*) have a high incidence of intestinal and urothelial neoplasms (Schacham et al, 1970; Pamukcu et al, 1977). The relatively high frequency of these lesions in cattle in the Scottish Highlands may be due to the regional abundance of this plant (Jarrett, 1980). Fed to conventional rats it induces ileal adenocarcinomas but it produces ileal sarcomas alone in germ-free rats (Sumi et al, 1981). The flavonoids kaempferol and quercetin may be the constituents most responsible for the mutagenic and carcinogenic effects of bracken fern (Pamukcu et al, 1980).

Cycasin, derived from the seed of the cycad tree (*Cycas circinalis*), and related compounds are the most selective and useful intestinal carcinogens yet discovered. In 1963 Laqueur and his colleagues were

investigating the high prevalence of amyotrophic lateral sclerosis among native islanders of Guam by examining their diet. The islanders obtained a form of edible flour from the cycad seed, which when fed to rats produced caecal adenocarcinomas in 2 but no neurological deficits. Cycad seed has other toxic effects and is carcinogenic to other organs (Laqueur et al, 1963). The active principal is beta D-glucosyl-oxyazoxymethane or cycasin, which is present in the seed at levels up to 2.3%. Its aglycone methylazoxymethanol (MAM) is mutagenic converting *Salmonella typhimurium* strains to histidine independence (Smith, 1966; Matsushima et al, 1979). It is also carcinogenic to the intestine, liver and kidney in rats (Zedeck et al, 1970).

Azoxymethane (AOM) and 1,2-dimethylhydrazine (DMH), synthetic analogues of cycasin, have proved to be potent and selective intestinal carcinogens in rats, mice and hamsters (Druckrey et al, 1967; Pozharriski et al, 1979). Two other naturally occurring hydrazines, 1,1-dimethylhydrazine found in tobacco and methylhydrazine present in edible wild false morel mushrooms (*Gyromitra esculenta*) are also carcinogenic to the intestine of rodents (Toth, 1982). Other synthetic analogues, 1-methyl-2-butylhydrazine, trimethylhydrazine and methyl-azoxybutane have similar properties (Druckrey, 1970; Toth, 1982).

Hydrazo-, azo- and azoxyethane are also potent carcinogens but with completely different target organs (thymus, breast and brain). They are not intestinal carcinogens (Druckrey and Lange, 1972; La Mont and O'Gorman, 1978).

The various cycasin derivatives and analogues produce different tumour distributions depending on the species and strain of rodent. For some compounds a single exposure may suffice in some strains (Hirono et al,

1968; Druckrey and Lange, 1972; Zedeck and Sternberg, 1974; Ward, 1975). For others a long course of oral administration or injection may be necessary. The most popular model has involved weekly subcutaneous injections of DMH (10-30 mg/kg/week); but azoxymethane is equally effective and does not require adjustment of pH before injection. Manipulation of the dose and duration of administration of these two substances can produce an almost 100% large bowel tumour yield with an acceptably low incidence of toxic side effects and extra-intestinal tumours in rats and mice (Sunter et al, 1978; Toth, 1982).

Metabolism and Mechanism of Action

Cycasin is a powerful carcinogen in conventional rats but not in germfree rats (Laqueur et al, 1963). By contrast, MAM is equally potent in both conventional and germfree animals. Intestinal bacteria possess the beta-glucosidase capable of liberating the active aglycone MAM from the conjugate cycasin (Figure 3.1) (Laqueur, 1965). Neither DMH nor AOM induce tumours at injection sites as they also require conversion to the active metabolite MAM. Druckrey's original proposal that both were metabolised to MAM in the liver was later supported by chromatographic studies carried out after the injection of C^{14} -DMH (Fiala, 1975, 1977). DMH appears to be activated through the following sequence: DMH- \rightarrow azomethane- \rightarrow azoxymethane- \rightarrow MAM (Jacobs et al, 1981). Other carcinogenic hydrazines may undergo a similar activation process in vivo.

MAM is unstable except in acetate form and is quickly converted to the alkylating agent methyl diazonium, which in turn decomposes spontaneously to release the highly reactive carbonium ion. Formaldehyde is a byproduct of this reaction, which may account for some of the toxic properties of the cycasin group.

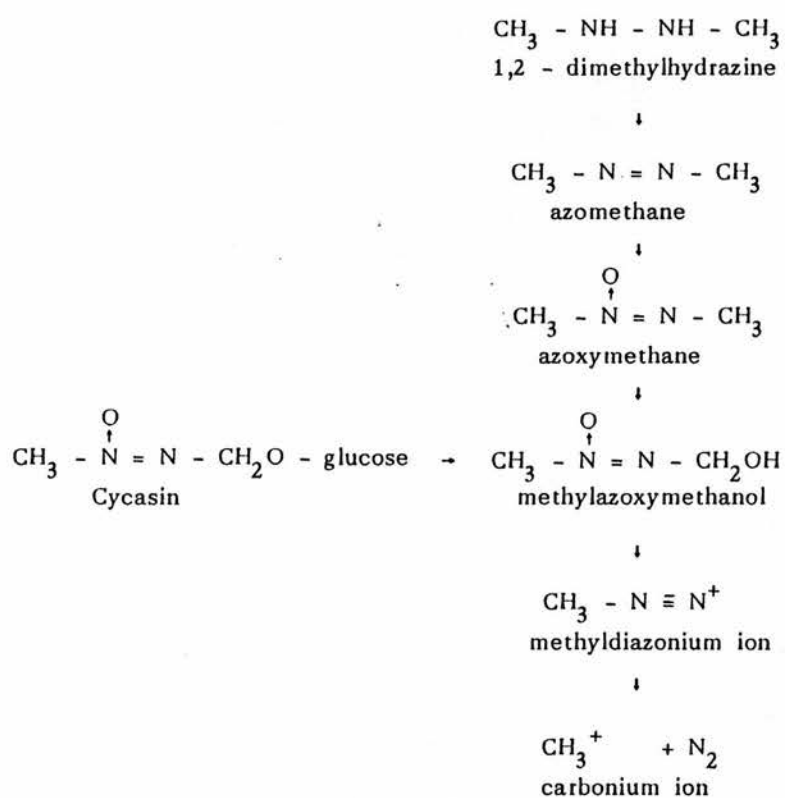


Figure 3:1 Metabolic pathways of cycasin, dimethylhydrazine and azoxymethane.

Studies with inhibitors reveal useful information on the activation mechanisms for carcinogens. Disulfiram and related compounds that contain a carbon disulphide moiety depress DMH-induced neoplasia (Wattenberg, 1975) probably by inhibiting the N-oxidation of azomethane and the hydroxylation of azoxymethane to MAM (Fiala, 1977) (Figure 3.1). Disulfiram also reduces intestinal carcinogenesis induced by the bracken fern derivative quercetin (Pamukcu et al, 1977). Pyrazole acts at other loci: azoxymethane hydroxylase as well as the alcohol dehydroxylase activation of MAM (Fiala et al, 1978; Notman et al, 1982). Selenium may act at one or more of these loci to cause inhibition of DMH-and AOM-induced neoplasia (Jacobs et al, 1981; Banner et al, 1982).

Both DMH and AOM at low dose induce tumours in rats that are virtually confined to the colon, but as the dose increases, tumours appear in the duodenum and proximal jejunum. Weisburger (1971) proposed that MAM was conjugated in the liver and secreted in bile as an inactive glucuronide which passes through the small bowel intact. Exposure to bacterial beta-glucuronidase in the colon would release the active carcinogen. Higher doses might overwhelm the hepatic conjugation mechanism resulting in biliary secretion of active MAM and the development of proximal small bowel tumours. This elegant hypothesis has been undermined by subsequent studies suggesting that carcinogenic metabolites reach the bowel epithelium via the bloodstream (Zedeck, 1978). Radioactivity is detectable in colonic DNA within an hour of ^3H -DMH administration to rats, and less than 1% of a different isotopic label (^{14}C) can be recovered from bile within 24 hours of injection (Pozharriski et al, 1979). Furthermore, colon sequestered from the faecal stream remains susceptible to carcinogenesis following subsequent administration of AOM (Campbell et al, 1975; Terpstra et al,

1981) and bile diversion to mid small bowel does not prevent the development of duodenal tumours following ingestion of azoxymethane (Williamson et al, 1979).

The organs affected by these carcinogens depend on the route of administration and the age of the animal. For example, the addition of DMH to drinking water of rats produces hepatic angiomas (Druckrey, 1970), while azoxymethane given to neonatal rats produces brain and kidney tumours (Druckrey and Lange, 1972) and cycasin given to pregnant rats causes gliomas and mesenchymal jejunal tumours in their offspring (Spatz and Laqueur, 1967). The only common extraintestinal tumour in older rats given parenteral AOM or DMH is squamous carcinoma of the external auditory canal (Ward et al, 1973a).

Organospecificity might depend on tissue-specific enzymes (e.g. alcohol dehydrogenase) capable of catalysing the production of the ultimate carcinogen (Grab and Zedeck, 1977). Insusceptible tissues might detoxify or simply fail to bind circulating carcinogenic metabolites (Fiala, 1977).

It is likely that chemical carcinogens initiate malignant transformation by structural modification of DNA producing irreversible changes in the cellular genotype (Cooper et al, 1978). Initiation appears to involve alkylation of nucleic acid bases. Methylation at various sites of the guanine molecule has been detected by chromatography soon after the administration of DMH and MAM to rats and mice (Shank and Magee, 1967; Hawks and Magee, 1974; Rogers and Pegg, 1977; Zedeck and Brown, 1977). The commonest alkylated bases found in bowel mucosa are 7-methylguanine and O⁶-methylguanine which are also present in the kidney and liver. However the degree of methylation in various tissues does not correlate with the incidence of ultimate tumour development (Hawks and Magee,

1974; Pozharriski et al, 1975; Rogers and Pegg, 1977). Nevertheless nucleic acid base methylation occurs to a greater extent in a strain of mouse that is susceptible to DMH-carcinogenesis (ICR) than in another strain that is not (C57) (Cooper et al, 1978).

The efficiency of DNA repair processes in various tissues may govern the organospecificity of chemical carcinogens. The mechanism for excising the methylated base O⁶-methylguanine may be deficient in target tissues (Pegg, 1977).

Pathology

Cytokinetic changes

Experimental tumours induced by cycasin analogues and direct acting carcinogens such as MNNG bear a close histological resemblance to human intestinal neoplasms (Ward et al, 1973a; Ward, 1974; Pozharriski et al, 1975).

After exposure to cycasin analogues a triphasic response can be identified in the bowel epithelium. Acute cell destruction and repair gives way to epithelial hyperplasia and eventual neoplasia. The initial reaction of gut mucosa to carcinogen treatment is an inhibition of DNA and RNA synthesis evident within one hour (Zedeck et al, 1970). Cell damage indicated by nuclear pyknosis and karyorrhexis is found in the region of the crypt where actively proliferating cells are found. Prominent necrosis of crypt cells is seen in both large and small bowel mucosa and is extensive within 6 hours (Zedeck et al, 1970; Sunter, 1980; Sunter et al, 1981). The distribution of acute mucosal damage appears to parallel the distribution of tumours which arise with continued carcinogen administration (Zedeck et al, 1977). In rodents this severe cytotoxic effect lasts about 48 hours and is

accompanied by a transient depression of crypt cell production (Lamont and O'Gorman, 1978; Zedeck, 1978).

The acute necrotic phase is followed by a prominent compensatory proliferative response (Deschner, 1978) which becomes evident at 4 days and is curtailed by about 7 days (Wright, 1983). This recovery is succeeded by a long latent period during which a further gradual increase in cell proliferation can be detected as early as one month after starting DMH administration (Springer et al, 1970; Thurnherr et al, 1973; Richards, 1977; Chang, 1978). This mucosal hyperplasia increases with time up to and beyond the development of tumours (Wright, 1983). The intestinal mucosal kinetic changes during cycasin group carcinogenesis are considered in more detail in Chapter 5.

Biochemical changes

Changes in goblet cell mucin secretion have been observed in apparently normal mucosa of human colon bearing tumours elsewhere. There is an increase in sialomucin production and a corresponding decrease in sulphomucins (Filipe and Branfoot, 1974; Shamsuddin et al, 1981). In the rat, marked increases in the activity of six enzymes involved in sialic acid metabolism have been detected in azoxymethane-induced tumours (Corfield et al, 1983). Changes in the activity of two of these enzymes were also found in the non-malignant mucosa of the same animals. This feature of altered mucin histochemistry in colonic mucosa susceptible to malignant transformation prompts further investigation which could lead to the development of useful techniques for the early detection of human colonic cancer (Veh et al, 1982). Other biochemical changes common to human and experimental colon cancer include reductions in carbohydrate content and

glycosyltransferase activity (Kim et al, 1974; Freeman et al, 1978a; Lamont and O'Gorman, 1978), and selective accumulation of non-histone nuclear proteins which may be associated with aberrations of nuclear function (Boffa and Allfrey, 1976; Boffa et al, 1980).

Morphological changes

The earliest changes are revealed by scanning electron microscopy of the colonic mucosa at about 4 weeks after starting the carcinogen. There is distortion of the epithelial cells with superficial protruberances accompanied by progressive destruction of the overlying mucous barrier (Toth and Malick, 1976; Barkla and Tutton, 1977). Light microscopy shows depletion of goblet cells and areas of local glandular hyperplasia (Springer et al, 1970; Thurnherr et al, 1973). Abnormal cells with basophilic cytoplasm and hyperchromatic nuclei, absent normal vacuoles and mucous droplets, accumulate in the upper part of single crypts. They can incorporate thymidine and resemble undifferentiated stem cells (Chang, 1978, 1980). These foci have been found as early as 22 days after exposure to carcinogen and become increasingly common over the next 2 months, progressing to severe dysplasia and carcinoma in-situ detectable from 12 weeks onwards (Thurnherr et al, 1973; Deschner, 1974; Maskens, 1976; Richards, 1977). Macroscopic tumours are first seen at about 15 weeks (Thurnherr et al, 1973; Maskens, 1976).

Sunter and colleagues (1978) have introduced a pathological classification of experimental tumours - (a) Adenomas: macroscopically sessile or pedunculated showing a tubular or tubulovillous structure, well differentiated with no evidence of invasion through the muscularis mucosae. (b) Group I carcinomas: macroscopically similar to adenomas but invading

through the muscularis mucosae. (c) Group II carcinomas: the commonest tumours, moderately well differentiated invasive adenocarcinomas. Macroscopically they appear as ulcerated polyps or plaques often with a peripheral rim or shoulder of adenomatous tissue suggesting origin from a non-invasive neoplasm. (d) Group III carcinomas: all poorly differentiated, highly invasive, metastasising widely with a variety of macroscopic and histological appearances. They have no apparent origin from adenomatous lesions. Some are mucinous, characterised by "signet ring" cells and most commonly found in the duodenum and ascending colon (Ross, 1982).

Tumours are often multiple, numbers increasing with time and dose of carcinogen. Their growth patterns can be described by a "Gompertz curve" (i.e. a short initial doubling time that lengthens with time. Maskens, 1976). Animals given sufficient doses of AOM or DMH develop lymphatic and transcoelomic metastases (Pozharriski et al, 1979). Unlike human colonic cancer liver metastases are very uncommon. Experimental colorectal cancers present with weight loss, rectal bleeding or abdominal distension (Williamson et al, 1980a, 1982). Rectal growths may prolapse through the anus. Intestinal obstruction generally occurs late and usually results from intussusception rather than annular constriction (Williamson et al, 1980a).

Although there are many more points of similarity than of dissimilarity between human and experimental colorectal tumours (Ross, 1982), there are certain flaws in the experimental model. Small bowel cancers, mucinous cancers and multiple cancers are more frequent in the animal model, which may merely reflect the degree of the carcinogenic stimulus. Experimental tumours can develop osseous metaplasia and frequently develop in close association with lymphoid follicles. These features are hardly ever seen in man (Ross, 1982; Williamson et al, 1982).

The caecum is a common site in man but appears relatively resistant to chemical carcinogenesis (Pozharriski, 1975).

CHAPTER 4
THE AETIOLOGY OF COLORECTAL CANCER

A. HEREDITY

Epidemiological studies revealing that populations who migrate from low-risk countries to high-risk countries develop increased susceptibility highlight the importance of environmental rather than genetic factors in large bowel cancer. On the other hand children of patients with familial polyposis coli have a 50% risk of inheriting the disease, which has an almost 100% chance of progressing to malignancy (Asman and Pierce, 1970; Bussey, 1975). Between these two extremes, there have been reports of families in which the risk of colorectal cancer in first degree relatives is greater than in the general population (Woolf, 1955; Lynch and Krush, 1967; Dunstan and Knaggs, 1972). Identically sited colonic cancers have been found in monozygotic twins (Whitehead, 1980). Lovett (1974, 1976) found that 26% of 209 patients with colorectal cancer had first degree relatives affected by the disease. The death rate from large bowel cancer in these relatives (10.9%) was three times that expected in the general population (3%). Patients with positive family histories tended to present younger, and often had associated adenomas, synchronous and metachronous cancers. Duncan and Kyle (1982) found 16% of a series of 50 patients, presenting in a very high-risk area (North East Scotland), with a positive family history compared with 2% of a matched control group. They found no association with age, adenomas or multiple tumours. Lynch et al (1977) found positive family histories to be more common in patients with tumours of the proximal colon.

The pattern of inheritance in such families is unclear. Lovett (1976) found that spouses of affected patients also had an increased risk of large bowel cancer, suggesting that the genetic predisposition may be modified by the environment. However, a study of over 1,000 married couples in Sweden

showed no increased risk in husbands or wives of affected patients (Jensen et al 1980).

The existence of families particularly susceptible to the disease emphasises the importance of taking a detailed family history. Indeed the identification and screening of relatives at risk may be life-saving.

Polyposis syndromes

"It is probably correct to say that polyposis is the most clearly defined precancerous disease known to medicine" - Professor J C Goligher, 1984.

Polypoid disease of the colon was first described by Menzel in 1721. In 1882 Cripps reported multiple rectal polyps in a brother and sister, one of whom had also developed rectal carcinoma. Smith (1887) found a third member of this family of six who had developed polyposis and died from colonic carcinoma at the age of 27. Hanford (1890) described a 30 year old woman in whom malignant degeneration had occurred in previously benign polyposis. Despite these early observations it was not until 1925 that Lockhart-Mummery fully identified and defined the condition of familial polyposis coli.

Familial polyposis coli is a hereditary disease characterised by the appearance in early adult life of large numbers (100-5000) of adenomatous polyps studded throughout the large bowel. Adenomas may also occur in the stomach, duodenum and ileum (Hamilton et al, 1979; Ranzi et al, 1981). If left untreated, the risk of colorectal cancer is almost 100% (Asman and Pierce, 1970; Bussey, 1975). Familial polyposis represents only a minute proportion of all large bowel cancer cases, but affected families are probably distributed throughout the world (Hill, 1982). It is carried by an autosomal dominant gene with a high degree of penetrance and affects both

sexes equally. Propositus cases usually develop symptoms in their late twenties or early thirties (Asman and Pierce, 1970; Bussey, 1975), and 50% will have developed at least one carcinoma before their fortieth birthday (Bussey, 1975). The importance of fully investigating families in which the disease is found is illustrated by Bussey (1975), who has found that two-thirds of all new patients presenting with symptoms already have carcinoma. On the other hand only 9% of affected individuals traced through family studies have cancer (Lovett, 1974).

Surgical opinion is divided about the most appropriate treatment for polyposis. Panproctocolectomy with ileostomy is the most logical approach, completely removing the affected organ and the risk of colorectal cancer, but it leaves the patient, who may have had no symptoms, with the permanent handicap of an ileostomy. Most surgeons argue that excision of the rectum is unnecessary and recommend subtotal colectomy and ileorectal anastomosis with six-monthly review and destruction of any residual or recurrent polyps thereafter. There have been sporadic reports of regression of rectal polyps following this latter procedure (Hubbard, 1957; Cole and Holden, 1959; Shepherd, 1971; Bussey, 1975). This phenomenon has been attributed to various factors; an inhibitory effect of ileal contents, loss of growth-promoting factors present in the intact colon or reduced blood supply to the rectum (Cole and Holden, 1959; Cole et al, 1961). The reported risk of cancer developing in the retained rectum varies from 6.5 - 59% (Morson, 1978; Moertel et al, 1970). These wide discrepancies between series may reflect differences in age at operation, the length of the retained segment, and the incidence of carcinoma in the resected specimen (Gingold and Jagelman, 1981).

In 1951, Gardner described a large family affected with polyposis coli

in which a large number of deaths had occurred from colonic cancer. He noted a wide variety of extraintestinal "surface" tumours which were present in a number affected with either polyposis or cancer. The nature of these tumours was not recorded, but they were distributed mainly around the head and upper body. He inferred that these tumours showed the same pattern of inheritance as the polyposis. The term Gardner's syndrome now applies to any case of intestinal polyposis associated with osteomas of the skull and mandible, multiple epidermal cysts and soft tissue tumours of the skin, desmoid tumours arising from the mesentery or previous abdominal incision, mesenteric fibrosis, dental anomalies and thyroid tumours (Bussey, 1975). Other rare associations include malignant tumours of the central nervous system (Turcot et al, 1959) and alopecia and nail dystrophy (Cronkhite and Canada, 1955). All these syndromes carry a high risk of colonic cancer and there may also be an increased risk of periampullary cancer (Jones and Nance, 1977; Pauli et al, 1980). Chromosomal aberrations have been observed in lymphocytes and fibroblasts cultured from patients with Gardner's syndrome (Gardner et al, 1982). The rate of sister chromatid exchange in these cells may be an early index of whether or not a family member actually has the syndrome.

Juvenile polyposis is determined by an autosomal dominant gene and characterised by the early appearance of hamartomatous polyps usually at about 6 years of age (McColl et al, 1964). Symptoms may appear in early childhood and include rectal bleeding, diarrhoea and anaemia. Polyps are usually found in the large bowel, bleed easily and are often shed and passed spontaneously (McColl et al, 1964; Veale et al, 1966). This condition was originally regarded as benign but more recently it has been considered to carry a very slight risk of malignancy (Morson, 1978).

In their original reports both Peutz (1921) and Jegher et al (1949) suggested that the gastrointestinal polyps that accompany the skin and mucosal pigmentation of the Peutz-Jaegher's syndrome might undergo malignant degeneration. There is little evidence to support this contention although intestinal cancer has occasionally been reported in association with the syndrome (Reid, 1974; Morson, 1978).

B. ADENOMA

Polyps, defined as any visible protrusion from the colorectal mucosa are thought to occur in 20%-50% of older adults (Chapman, 1963; Fenoglio and Lane, 1975). About 90% of all polyps are hyperplastic and only 10% neoplastic or adenomatous (Fenoglio and Lane, 1975). In hyperplastic polyps, the balance of cell division in the crypts and exfoliation from the surface is disturbed, resulting in the accumulation of mature cells in the upper part of the crypt and on the mucosal surface. Crypts are elongated and dilated and contain fewer goblet cells and more fully differentiated absorptive cells than usual. Cell division as demonstrated by thymidine labelling is confined to the lower part of the crypt (Lane et al, 1971; Lane, 1976). The normal mechanism for repression of DNA synthesis remains intact and hyperplastic polyps are not precancerous (Morson, 1974).

By contrast, cell differentiation is impaired in adenomas and cell division appears unrestricted, with mitotic figures appearing at all levels, even on the mucosal surface. The lesions bear the hallmark of true neoplasia (Fenoglio and Lane, 1975). The incidence of malignancy, defined as invasion through the muscularis mucosae, is about 1-5% in all adenomas (Morson, 1974; Lane, 1976; Gillespie et al, 1979).

The true incidence of adenomas is difficult to ascertain accurately but they have been reported in 24-39% of adult colons, increasing in prevalence with age (Blatt, 1961; Arminski and Maclean, 1964). They are commoner in men (Ekelund, 1963; Day and Morson, 1978). More than half of patients with adenomas have multiple lesions and 2% have more than 5 adenomas (Gillespie et al, 1979). The predominant histological growth pattern is the tubular adenoma which is usually pedunculated and accounts for about 75% of all adenomas. At the other end of the spectrum of tissue architecture

the villous adenoma (papilloma) occurs in 10%. The remainder consists of adenomas containing features of both these extremes; the so-called tubulovillous pattern (Morson, 1974; Shinya and Wolff, 1979).

Evidence of these three patterns of adenomatous change is detected with roughly equal frequency in colorectal cancers (Morson, 1974). As a much smaller proportion of all adenomas are villous as opposed to tubular, villous tumours would seem to have a greater malignant potential. Indeed malignancy is found much less commonly in tubular adenomas than in villous lesions (Morson, 1974; Gillespie et al, 1979; Shinya and Wolff, 1979). Two factors may contribute to the greater malignancy rate in villous adenomas. Firstly, size may be the most important index of malignant potential. Malignancy is rare in adenomas of less than 1 cm in diameter; it is found in 10% of tumours of 1-2 cm diameter and in 50% of tumours of greater than 2 cm (Morson, 1974). Tubular adenomas rarely grow to more than 3 cm, and 33% of those greater than 2 cm are malignant (Morson, 1974, 1978). On the other hand villous tumours tend to grow very much larger (up to 10 cm) (Grinnel and Lane, 1958), and 58% of those greater than 2 cm are malignant (Morson, 1974, 1978).

Dysplasia is a second major indicator of malignant potential. Mild dysplasia is associated with a low incidence of carcinoma while the features of severe dysplasia (nuclear enlargement, pleomorphism, loss of polarity, stratification of nuclei, increased normal and abnormal mitotic figures and decreased differentiation) are associated with a 33% incidence of malignancy. Severe dysplasia is rare in small polyps and more common in the larger lesions particularly those of the villous pattern (Morson, 1974; Day and Morson, 1978). Tubulovillous adenomas have an intermediate malignant rate which is closer to that of the villous than that of the tubular type.

There is an accumulating wealth of evidence to support the concept that most, if not all, large bowel cancers arise in pre-existing adenomas: the adenoma-carcinoma sequence. Few epidemiological studies have been carried out but the available evidence suggests a positive correlation between the geographical prevalence of adenomas and the incidence of large bowel cancer (Morson, 1978). The average age of presentation with adenomas is 4-7 years lower than that for carcinoma (Enterline, 1976; Day and Morson, 1978). As polyps can be clinically silent for a very much longer period than carcinomas this reported age difference may be a considerable underestimation. Autopsy and colonoscopy studies indicate that the anatomical distribution of polyps is similar to that of carcinomas; polyps are most commonly found in the distal descending or sigmoid segments (Ekelund, 1963; Gillespie et al, 1979; Shinya and Wolff, 1979). In a large series of 1,049 adenomas endoscopically removed from 620 patients, Gillespie and co-workers (1979) found that 94% of those containing invasive cancer occurred in these segments.

Fourteen per cent of a series of 1,961 large bowel cancers examined at St Mark's Hospital contained contiguous benign tumour tissue (Morson, 1974). The incidence of adenomatous change ranged from 60%, if the carcinoma was confined to the submucosa, to only 7% if the bowel wall had been breached. As cancers grow they appear to displace adjacent adenoma, and benign tissue is rarely found in cancers of greater than 2 cm (Lane, 1976). About a third of all colorectal cancer specimens have one or more adenomas in the specimen (Day and Morson, 1978). In these patients the risk of subsequent development of metachronous tumour is doubled compared to large bowel cancer patients overall (Bussey et al, 1967; Morson, 1974). Heald and Bussey (1975) found at least one adenoma in 75% of all specimens

of synchronous cancers. Circumstantial evidence for the polyp-cancer theory comes from the fact that de-novo carcinomas (< 5 mm) or foci of microcancer have never been demonstrated without pre-existing adenoma even in the non-polypoid mucosa of familial polyposis patients (Fenoglio and Lane, 1975; Lane, 1976).

Perhaps the most compelling evidence for the adenoma-carcinoma sequence comes from the study at the University of Minnesota Cancer Detection Centre involving 85,000 patient years (Gilbertson, 1974). Regular proctosigmoidoscopy with destruction of all polyps reduced the expected incidence of rectosigmoid cancer by 85%. Moreover, those cases found were all early cancers.

Morson (1974, 1978) has proposed that the progression from adenoma to carcinoma may take many years. Many polyps never become malignant and even villous adenomas may remain benign for more than 20 years.

C. DIET

".....It is ironic that, while the population of the industrial world is enjoying unprecedented health and longevity, there is a prevailing concern that our technological society is placing upon us an intolerable burden of cancer. Added to this concern is the growing realisation that what we do to ourselves may be more hazardous than what is done to us by our environment" - Dr Sidney Weinhouse, 1983.

The geographical distribution of large bowel cancer correlates more than any other cancer with industrialisation and affluence (Chapter 2). It is probable that the most striking difference in the environment of a high-risk wealthy country compared to that of a low-risk poor country would be in the diet. Not surprisingly, therefore, the influence of diet in the aetiology of colorectal cancer has been the focus of much attention.

Few foods contain substances which are directly carcinogenic to large bowel mucosa. In the Pacific Islands and in other tropical and subtropical regions, all parts of the cycad plant (*Cycas circinalis*), have been used either as staple food or for medicinal purposes (Whiting, 1963). Raw cycad extracts are highly toxic. The active principle of the cycad seed is beta-D-glycosyloxyazoxymethane or cycasin, which is a compound of glucose and an aglycone, methylazoxymethanol (MAM). MAM is a direct mutagen (Smith, 1966; Matsushima et al, 1979) and carcinogenic to the colon, duodenum, liver and kidney in rats (Zedeck et al, 1970). Populations that customarily eat cycad extracts have a high incidence of amyotrophic lateral sclerosis (Laqueur et al, 1963) but a low incidence of colorectal cancer. Presumably the elaborate methods of preparation needed to make them edible destroys their carcinogenicity.

Aflatoxin B₁, a fungal metabolite which may contaminate peanut butter, is weakly carcinogenic to the intestinal tract (Newberne and Rogers, 1973; Ward et al, 1975). Another naturally occurring intestinal carcinogen

is methylhydrazine which causes colonic adenomas and carcinomas in Syrian hamsters and makes up 11 µg/g of *Giromitra esculenta*, an edible false morel mushroom (Toth, 1982). Tobacco and tobacco-smoke contain 1,1-dimethylhydrazine, another possible intestinal carcinogen (Toth, 1982). Bracken fern, which contains quercetin and other flavonoid carcinogens that are not inactivated by cooking, is a popular salad green in some areas of the USA and Japan (Pamakcu et al, 1977). The high prevalence of colon cancer in Scotland may be related to the abundance of bracken fern in certain areas, as milk from cows grazing on the plant may be carcinogenic to the intestine (Jarret, 1980).

The preparation of some foods may render them potentially carcinogenic (Doll, 1978; Reddy et al, 1980). Mutagens (presumptive carcinogens) have been demonstrated in the charred surface of grilled or fried meat and fish. These mutagens are thought to arise from protein pyrolysis and their active principle is an O-methylarylamine. Other methylarylamines (eg 3,2-dimethyl-4-aminobiphenyl) produce colon cancer in rats (Reddy et al, 1980). Browning reactions desirable for producing the flavour and aroma of cooked foods (not just meats) have been shown to produce mutagenic activity. Nitrites used in the curing of meat could readily generate N-nitroso compounds, perhaps enough to escape absorption in the upper alimentary tract (Lijinsky, 1977).

Many substances added to food either intentionally as preservatives, colourants and flavouring, or unintentionally as pesticides, have initiated cancer in the laboratory. Some (eg DDT and saccharin) produce tumours only in special circumstances which are of dubious relevance to the aetiology of human cancer. Although large doses of these additives can cause cancer in small animals, it is too early to deduce the possible long term results of minute amounts in the human diet (Doll, 1978).

Apart from this small number of suspected direct intestinal carcinogens, it is unlikely that any major dietary constituent is carcinogenic to the human large bowel. It is more likely that diets containing different proportions of various foods deliver different compositions of luminal substrates to the enormous bacterial population of the large bowel (Heaton, 1977). Bacterial metabolism of food residues and alimentary secretions might produce cocarcinogens or carcinogens in quantities modified by the composition of the diet.

Evidence regarding the role of various dietary factors in colorectal cancer is based on both epidemiological and experimental data. Neither source is infallible. National dietary statistics often suggest relationships between nutrient intake and cancer incidence which are not confirmed by case-control studies (Moskovitz et al, 1979; Hunter et al, 1980; Enstrom, 1981). Dietary manipulations of experimental tumour models often fail to allow for changes in total calorie intake or body weight (Heaton and Williamson, 1978). Nevertheless, the balance of the available evidence supports the concept that diet is the major influence in the aetiology of cancer of the large bowel. The individual dietary constituents which have been implicated as important factors in colorectal carcinogenesis are reviewed below.

Cancer promoting agents

Fat

In 1967 Wynder first proposed that the risk of colorectal cancer was related to the total dietary intake of fat (Wynder and Shigematsu, 1967; Wynder and Reddy, 1974). Subsequently several international studies have confirmed a positive correlation at the national level between dietary fat

intake and large bowel cancer incidence (Drasar and Irving, 1973; Armstrong and Doll, 1975; Correa, 1981). However, the validity of these data is weakened when the dietary habits of subpopulations with varying incidences of the disease within countries are examined. Correlations between large bowel cancer rates and fat intake in different areas within the United States, India and Scandinavia are essentially zero (Enstrom, 1975; Heaton, 1977; Enstrom, 1981). Colorectal cancer rates in Mormons and Americans of Japanese or Mexican extraction are half that of the overall population, while their dietary fat intakes are no different from the national level (Enstrom, 1981). Eskimos consume an enormous proportion of fat without any particular susceptibility to the disease (Cruse et al, 1979). Dietary surveys have so far failed to reveal any difference in fat consumption between patients with large bowel cancer and matched controls (Heaton, 1977).

There is strong experimental evidence for a cancer-promoting property of dietary fat. High fat diets enhance experimental colorectal carcinogenesis induced by dimethylhydrazine, azoxymethane, methylazoxymethanol, or methylnitrosourea (Reddy et al, 1974a, 1976a,b, 1977a; Nigro et al, 1975; Wilson et al, 1977; Bull et al, 1979). It seems unimportant whether or not the extra fat is of animal or vegetable origin (Rogers et al, 1980; Reddy, 1981). Diets deficient in lipotropes (methionine or choline) enhance dimethylhydrazine-induced carcinogenesis in rats (Rogers and Newberne, 1973).

The mechanism by which dietary fat might promote colorectal cancer is uncertain. It seems unlikely that it has a direct effect, but its proportion in the diet may alter the metabolic activity of faecal microflora (Reddy et al, 1980) or increase faecal excretion of cholesterol and acid and neutral

sterols which may themselves be potent cocarcinogens. Fat may modify the metabolism of carcinogens by affecting mucosal enzyme systems (Reddy et al, 1977a). Kollmorgen et al (1979) found that a high fat diet inhibited lymphocyte function in the rat and suggested that a high fat intake might reduce the efficiency of immunological defence against bowel cancer.

Cholesterol

Since Western populations share a susceptibility to both ischaemic heart disease and colorectal cancer, it is reasonable to postulate that these two conditions share common aetiological factors (Rose et al, 1974; Broitman, 1981). The most striking common factor may be cholesterol. There is strong correlation at the population level between mean blood cholesterol concentration and ischaemic heart disease and in individuals the serum cholesterol level is a powerful indicator of coronary artery disease risk (Rose et al, 1974). Evidence relating cholesterol to colorectal cancer is more controversial (Wynder, 1975; Feinleib, 1983). Liu et al (1979), using food disappearance data from 20 industrialised countries, found that cholesterol intake correlated with colon cancer incidence more than with fatty acids or fibre. Patients with colorectal cancer may excrete more cholesterol metabolites than matched controls (Reddy and Wynder, 1977), and inability to degrade dietary cholesterol has been proposed as a risk factor (Cruse et al, 1979). The serum cholesterol level does not correlate well with large bowel cancer risk. Rose et al (1974), in a six-country prospective survey, found only 90 individuals with ischaemic heart disease who ultimately developed large bowel cancer. Almost two-thirds of these individuals had serum cholesterol levels below the expected figure. These authors were the first to suggest that dietary measures designed to lower

serum cholesterol after the diagnosis of heart disease might increase faecal cholesterol and bile acid excretion and thereby contribute to the development of bowel cancer. Alternatively, serum cholesterol level is a poor index of the amount of cholesterol present in the bowel lumen and patients with malignant disease might have fluctuating blood levels (Broitman, 1981).

Animal experiments involving increased dietary cholesterol have produced conflicting results: enhanced, unchanged or depressed carcinogenesis (Reddy and Watanabe, 1979; Broitman et al, 1982; Cohen et al, 1982). Interestingly, a cholesterol-free elemental diet decreases experimental colorectal carcinogenesis. If cholesterol is added to this elemental diet, tumour yields return to control levels (Cruse et al, 1978a). The administration of cholestyramine and candicidin or the addition of polyunsaturated fat to the diet, manoeuvres which shunt cholesterol from the blood to the bowel lumen, all produce enhanced carcinogenesis (Nigro et al, 1973, 1977; Asano et al, 1975; Broitman et al, 1977). Similarly, Clofibrate, a drug designed to reduce serum cholesterol, may be associated with a subsequent increased risk of colorectal cancer (Broitman 1981)

Protein

Colorectal cancer incidence is correlated with the consumption of animal protein equally or more closely than with fat (Drasar and Irving, 1973; Armstrong and Doll, 1975; Howell, 1975; Doll, 1978; Gregor et al, 1969). Beef consumption appears more closely related to large bowel cancer rates than pork, poultry or fish (Howell, 1975). Beef is a rich source of animal fat and populations consuming high fat diets also tend to eat large amounts of animal protein.

As with dietary fat, case control studies reveal unimpressive correlations between protein intake and large bowel cancer (Higginson, 1966; Wynder and Shigematsu, 1967; Wynder et al, 1969). However, Japanese migrants in Hawaii with the disease were two and a half times more likely than control subjects to have adopted a Western-style diet containing high levels of meat and legumes (Haenszel et al, 1973). High beef consumption might explain the high incidence of colorectal cancer in Argentina, a relatively poor and underdeveloped country, and might account for the extremely high colorectal cancer rates in New Zealand and Scotland (Doll, 1978). The disease is much less common in Finland than in Argentina or the USA. Finns consume as much fat as Argentinians or Americans but eat more dairy products and less meat (Reddy et al, 1980). Seventh Day Adventists who abstain from meat have a relatively low incidence of intestinal cancer (Phillips, 1975). By contrast, Mormons in Utah are among the highest beef consumers in America but still enjoy some resistance compared to their compatriots (Lyon et al, 1976).

In animals, alterations in dietary protein content have little or no effect on experimental colorectal carcinogenesis (Topping and Vissek, 1976; Vissek et al, 1978).

Alcohol

Although it has been accepted for over half a century that alcohol intake may be related to cancer of the upper gastrointestinal tract, evidence for a connection with large bowel cancer is flimsy (Doll, 1978). Some epidemiological studies in Britain, Norway and the USA have shown a correlation between the disease and alcohol consumption (Breslow and Enstrom, 1974). However, case-control studies and animal experiments have been mostly inconclusive.

A low consumption of beer could explain the relative resistance to intestinal cancer enjoyed by Mormons and Seventh Day Adventists (Enstrom, 1977). Dean et al (1979) carried out a retrospective study of the cause of death in 1,628 workers at a large Dublin brewery who received a free daily issue of stout. The incidence of rectal cancer in this group was twice that expected. The same team carried out a case control study in Dublin and London based on detailed investigation of patients' and controls' lifetime drinking habits. For long-term beer drinkers the relative risk for developing colon cancer was 1.77 with no increased risk of rectal cancer. A regular intake of spirits for at least 10 years was associated with a two-fold increase in the risk of cancer at both sites (Ward et al, 1983).

Cancer preventing agents

Fibre

Fibre can be bracketed with fat and protein as one of the three possible dietary factors in colorectal carcinogenesis which have received most attention from epidemiologists. The generic term "fibre" covers dietary components which are not metabolised by intestinal secretions. There are a variety of such substances including cellulose, hemicellulose, pectin and lignin, with different structures each having discrete physiological properties.

Half a century ago, Stocks and Karns (1933) examined the dietary habits of 450 patients with large bowel cancer and found no correlation with meat intake but a strong negative correlation with milk, wholemeal bread and vegetables. Burkitt (1971, 1976) observed that native populations of Africa who had a low incidence of colorectal cancer consumed much more dietary fibre than Western populations. He noted that the majority of

colonic cancers arise at sites of physiological stasis (caecum, sigmoid colon), and suggested that high fibre diets might protect against the disease by reducing luminal exposure to carcinogens. Fibre could produce this effect in several ways: by diluting intestinal contents, reducing transit time, adsorbing potential carcinogens or by altering bacterial action on carcinogen precursors (Heaton, 1977; Cummings, 1981; Kritchevsky, 1983).

There is a general lack of adequate data relating national fibre intake with cancer rate. Most major studies have found no convincing association between low fibre intake and increased cancer risk (Drasar and Irving 1973; Armstrong and Doll, 1975), but some have shown a weak correlation (Howell, 1975; Schrauzer, 1976). The relative insusceptibility of Finns to colorectal cancer compared to other European populations could be attributed to a relatively high fibre consumption (I.A.R.C., 1977).

The results of animal experiments are difficult to evaluate. Different strains are used, receiving varying basic diets and different carcinogens administered by diverse routes. Rats eating a fibre-rich diet appear to develop fewer tumours in response to dimethylhydrazine compared to rats on normal chow (3-5% fibre) (Barbolt and Abraham, 1978; Chen et al, 1978; Fleiszer et al, 1978, 1980). These diets were not isocaloric with controls and body weight was usually lower in the test group. However if dietary fibre content is increased without altering energy content, body weight is maintained, stool weight is increased and carcinogenesis is reduced (Wilson et al, 1977; Freeman et al, 1978b). Different types of fibre have different effects. Watanabe et al (1979) fed alfalfa, bran or pectin (15%) to rats given subcutaneous azoxymethane or intrarectal methylnitrosourea. Pectin and bran, but not alfalfa, reduced AOM carcinogenesis. Alfalfa actually enhanced MNU-induced carcinogenesis, while the other two substances had



no effect. Similarly a diet enriched with 6.5% citrus pectin enhanced dimethylhydrazine-induced tumour formation (Bauer et al, 1979), perhaps by increasing bacterial beta-glucuronidase activity or by binding bile salts in the colonic lumen thus damaging the mucosa (Cassidy et al, 1981). By contrast, Cruse et al (1978b) fed fibre at concentrations of 5-20% but found no protective effect against carcinogenesis induced by a very high dose of DMH. Carcinogenesis after azoxymethane was unaffected by a diet containing 40% fibre (Ward et al, 1973b).

Elemental diet

The commercially available liquid elemental diet "Vivonex" has been administered to rats with diverse results. Baker et al (1978a) found increased DMH-induced colorectal carcinogenesis. This effect, which was attributed to increased gut transit time, was abolished by the addition of methyl cellulose to the diet. On the other hand, Castleden (1977) observed a protective effect with Vivonex which was not related to any decrease in body weight. Colonic weight, total crypt cell population and proliferative parameters are all reduced in rats not receiving carcinogen fed on Vivonex (Janne et al, 1977). However, Heitman et al (1983) showed that the administration of a liquid elemental diet did not prevent the preneoplastic colonic mucosal hyperplasia normally seen after dimethylhydrazine administration.

Laxatives

Constipation, laxative ingestion and previous haemorrhoidectomy may all be commoner in colon cancer patients (Wynder and Shigematsu, 1967; Wynder et al, 1969). Experimental evidence regarding laxatives is not

striking, perhaps because of the difficulty of finding a suitable model: a rat eating a high-fat/high-protein/low-fibre diet, chronically constipated and ingesting laxatives on a regular basis. Magnesium sulphate does not affect colorectal carcinogenesis induced by 3-2'-dimethyl-4-aminobiphenyl despite profuse diarrhoea (Cleveland and Cole, 1969). The slight protective effect of dioctyl sodium sulphosuccinate on DMH carcinogenesis is probably due to an associated reduction in body weight (Karlin et al, 1980).

Vegetables

The low incidence of large bowel cancer in Seventh Day Adventists may be attributable to their vegetarian diet (Phillips, 1975). There is a clear negative correlation between dietary vegetable content and the disease (Graham et al, 1978). Brassica vegetables: cabbage, cauliflower, broccoli and sprouts inactivate polycyclic hydrocarbons in the gut and might thus interfere with carcinogen metabolism (Wattenberg, 1978; Wattenberg and Loub, 1978). Plant sterols, plentiful in vegetarian diets, partly inhibit carcinogenesis induced by N'-methyl-N-nitrosourea (Raicht et al, 1980). Protease inhibitors are abundant components of all seeds and have been found to inhibit tumour promotion both in vivo and in vitro (Yavelow et al, 1983).

Miscellaneous

Food contains a large number of natural and synthetic constituents which may inhibit carcinogenesis (Wattenberg, 1983).

Low levels of dietary selenium correlate well with large bowel cancer incidence throughout the world (Jansson et al, 1975; Schrauzer et al, 1977). Its addition to the diet of rats and sheep reduces the yield of colorectal

tumours in response to dimethylhydrazine, methylazoxymethanol (Jacobs, 1977; Jacobs, 1983), azoxymethane (Soullier et al, 1981), and bis-(2-oxopropyl) nitrosamine (Birt et al, 1982). The mechanism by which selenium exerts this effect is unknown (Banner et al, 1982).

Vitamin A (or its analogues) appears to have a similar protective effect in experimental tumour models (Rogers et al, 1973; Newberne and Suphakarn, 1977) although some studies have produced directly contradictory results (Narisawa et al, 1976). Vitamin C inhibits carcinogenesis in rats receiving DMH (Reddy et al, 1980) and may have a similar effect in patients with familial polyposis coli (De Cosse et al, 1975). Vitamin D has also been suggested as a tumour inhibitor which might explain the high incidence of colorectal cancer in populations exposed to the least amount of natural light (Garland and Garland, 1980).

Potassium may be a protective factor. Citizens of Seneca County, New York are particularly resistant to large bowel cancer, which has been attributed to the high concentration of potassium in the glacial Finger lakes (Jansson, 1982).

Endogenous factors

The importance of endogenous luminal constituents such as bile acids and bacteria in the aetiology of colorectal cancer is discussed in Chapter 8.

Nutrient intake

Intestinal mucosal cell kinetics are strongly affected by the presence or absence of food in the gut. Starvation and parenteral nutrition rapidly produce mucosal hypoplasia, whereas overeating leads to hyperplasia (Williamson, 1978). Mucosal atrophy develops rapidly distal to the site of a

proximal colostomy but is precisely reversed soon after continuity of the bowel is restored (Tilson et al, 1976; Terpstra et al, 1981). Directly or indirectly, luminal nutrients maintain the mucosal integrity of the intestinal tract (Hageman and Stragand, 1977). Total caloric intake may therefore be the crucial dietary variable in colorectal carcinogenesis (Heaton, 1977; Heaton and Williamson, 1978; Williamson, 1980).

In affluent populations satiety is generally achieved by eating large quantities of sweetened fatty foods, which are not only appetising to the Western palate, but also rich in calories and expensive. In poorer underdeveloped countries cheaper fibre-rich foods produce a similar sensation of satiety associated with a much lower calorie intake (Heaton, 1977). Alcohol also provides calories without leaving any residue. Western diets therefore have a high calorific value but are relatively low in fibre.

Calorific intake certainly affects carcinogenesis both in animals and man. Mice rendered obese by gold thioglucose are more prone to experimental mammary cancer than controls (Waxler et al, 1953). Decreased food intake depresses the development of many experimental tumours (Carroll, 1975; Clayson, 1975). Life insurance tables suggest that obesity is associated with an increased risk of the disease (Tannenbaum, 1940; Wynder and Shigematsu, 1967). This proposal has been strongly upheld by a long-term prospective study of 750,000 men and women in America in which large bowel cancer was found to be almost twice as common in those > 40% overweight than in those of normal weight. The risk was reduced compared to normals in the underweight (Lew and Garfinkel, 1979). The international incidence of large bowel cancer correlates with calorie intake (Armstrong and Doll, 1975).

D.INFLAMMATION

Ulcerative colitis

Colonic carcinoma superimposed on ulcerative colitis was first described in 1925 by Crohn and Rosenberg. Since then ulcerative proctocolitis has been regarded as an important precursor condition to large bowel cancer. Cancers arising in the presence of colitis account for less than 1% of the overall incidence (Riddell et al, 1978). About 1 in 10 of total colectomy or panproctocolectomy specimens resected for intractable colitis contain one or more previously undiagnosed adenocarcinomas (Kewenter et al, 1978; Van Heerden and Beart, 1980). The risk of cancer in ulcerative colitis depends on several factors, the most important of which is duration of symptoms. Bloody diarrhoea is a fairly striking symptom and its onset can usually be recalled with some accuracy (Devroede, 1980). There is a minimal risk up to ten years after the onset of the disease, increasing thereafter with time until at 30 years the risk is approximately thirty times that of the normal population (Lennard-Jones et al, 1977; Kewenter et al, 1978; Greenstein et al, 1979; Van Heerden and Beart, 1980). The extent and distribution of the disease may also affect the risk. In general, patients with left-sided colitis (distal to mid-transverse colon) develop cancer 10 years later than those with more extensive disease (Greenstein et al, 1979). However before the relatively recent introduction of the colonoscope, patients with left-sided colitis may have had more extensive disease undetected by barium enema, so figures comparing cancer risk with extent of disease might be inaccurate (Greenstein et al, 1979). The risk of cancer in ulcerative proctitis is probably minimal (Devroede, 1980), but there is a 9-12% incidence of carcinoma in the retained rectum within 25 years of subtotal colectomy and ileorectal anastomosis (Baker et al, 1978b;

Grundfest et al, 1981). Carcinoma has also been reported in the out-of-circuit rectal stump following subtotal colectomy and ileostomy (Lavery and Jagelman, 1982).

Early age of onset may be a sinister factor in its own right if only because it provides a longer period for cancer to develop (Edwards and Truelove, 1964; Greenstein et al, 1979; Devroede, 1980). There is no convincing data indicating whether the severity of the initial attack or the length of remission is related to risk (Devroede, 1980). There is, of course, a group of patients with a fulminating initial attack or whose symptoms become rapidly debilitating necessitating early panproctocolectomy, in whom the risk of cancer is unknown (Lennard-Jones et al, 1977).

Cancers superimposed on ulcerative colitis vary in several ways from ordinary colorectal cancer. The mean age of diagnosis is two decades earlier and multiplicity is more common (23-25% versus 3.5%) (Farmer et al, 1971; Kewenter et al, 1978; Brook, 1980; Van Heerden and Beart, 1980; Ritchie et al, 1981). Histologically they are more malignant and further advanced by the time of diagnosis, probably because their presence is often masked by symptoms of colitis (Van Heerden and Beart, 1980; Ritchie et al, 1981). They are more evenly distributed throughout the colon but the rectum is the commonest site (Riddell et al, 1978).

Despite these differences the prognosis in colitis cancer is similar to ordinary large bowel cancer particularly when matched stage for stage (Hughes et al, 1978; Ritchie et al, 1981).

Ulcerative colitis produces marked mucosal cytokinetic changes. Cell proliferation and cell migration are accelerated (Eastwood and Trier, 1973). The colonic crypt cell production rate appears to be increased not only in patients with active disease but also in those in clinical remission (Allan et

al, 1984). Cell division is no longer inhibited by phosphodiesterase inhibitors such as theophylline, particularly if the disease is of more than 10 years duration (Alpers et al, 1980). Morson and Pang (1967) described the appearance of overt cellular atypia in endoscopic biopsies and suggested that such changes should be regarded as premalignant. Patients with severe epithelial dysplasia have a 50% chance of concomittant carcinoma somewhere in the large bowel (Riddell et al, 1978) but neoplasia can develop in the absence of such changes (Evans and Pollock, 1972).

In animals the sulphated polysaccharide carageenan, derived from red seaweed, given orally produces blood and mucus in the stool along with pathological changes similar to ulcerative colitis (Marcus and Watt, 1969; Iatropoulos et al, 1975). Both degraded and undegraded carageenan enhance experimental carcinogenesis in response to azoxymethane, dimethylhydrazine or methylnitrosourea (Dahm et al, 1977; Watanabe et al, 1978). A single colonic adenocarcinoma has been found after undegraded carageenan alone (Watanabe et al, 1978).

Crohn's disease

Crohn's colitis was initially considered to carry no increased risk of large bowel malignancy and the finding of a carcinoma in a segment of inflamed colon often prompted revision of the diagnosis to ulcerative colitis (Lightdale and Sherlock, 1980). More recently it has become apparent that both large and small bowel carcinoma may develop in association with longstanding Crohn's inflammation (Greenstein et al, 1978). Crohn's enteritis is associated with an increased risk of small bowel cancer (Hoffman et al, 1977) and over 30 cases of adenocarcinoma arising in colonic segments affected by Crohn's disease have been reported (Warren and

Sommers, 1948; Greenstein et al, 1978; Lightdale and Sherlock, 1980). The risk of cancer in Crohn's colitis may be as high as 20 times normal (Weedon et al, 1973).

Large bowel Crohn's cancer resembles that seen in ulcerative colitis in the early age of diagnosis (50% under the age of 40), and the high proportion of tumours arising proximal to the splenic flexure (Lightdale and Sherlock, 1980). Epithelial dysplasia like that in ulcerative colitis has been described in two cases of granulomatous colitis complicated by carcinoma (Craft et al, 1981).

Other colitides

Neither appendicitis nor diverticulitis have any demonstrable role in the pathogenesis of colorectal cancer although they share the same geographical distribution and possibly the same aetiological factors particularly lack of dietary fibre (Moertel et al, 1974; Burkitt, 1971).

Longstanding chronic schistosomal colitis produces pathological features similar to those in ulcerative colitis (Ming-Chai et al, 1981) and is associated with increased large bowel cancer risk in China (Ming-Chai et al, 1980). Other chronic intestinal infestations such as amoebiasis have not been firmly linked with cancer.

E. INJURY

Radiation

Slaughter and Southwick (1957) were the first to suggest that therapeutic radiation might be associated with a risk of cancer developing within the irradiated field. Two of nine patients in whom this occurred had tumours of the large bowel. Since then there have been several case reports and small series reporting colorectal cancer following pelvic irradiation for benign and malignant gynaecological conditions (Palmer and Spratt, 1956; Castro et al, 1973; Qizilbash, 1974; Martins et al, 1980). Martins and colleagues compiled a series of 68 such patients from the literature. The mean age at diagnosis was 64 years and the interval between radiotherapy and cancer diagnosis ranged from 14 months to 33 years with a mean of 15.2. These tumours appear to carry a poor prognosis (Castro et al, 1973). Nearly all are rectal adenocarcinomas but colonic carcinomas and squamous carcinomas of the anal canal have been reported. Most conform to the criteria for radiation induced cancer laid down by Black and Ackerman (1965): a time interval of at least 10 years between exposure and cancer diagnosis and demonstrable radiation damage in the tissue adjacent to the tumour.

Radiation damage to the bowel produces symptoms of chronic proctitis in about 5-10% of cases and pathological features include ulceration, haemorrhage and necrosis (Martins et al, 1980). Healing may result in scarring and stricture formation. Such changes can occur from 2 months up to 30 years after radiotherapy. This chronic inflammation together with the mutagenic potential of X-rays may account for their apparent carcinogenicity.

There is a suggested association between gynaecological cancer and large bowel cancer (Schottenfield et al, 1969; Adelstein et al, 1979) which may explain the incidence of rectal cancer in patients who have had radiotherapy for cervical carcinoma. On the other hand, the true risk of cancer in the irradiated rectum may be obscured by the generally poor prognosis in gynaecological cancer. Moreover, pelvic irradiation for benign conditions and spinal irradiation for ankylosing spondylitis may also produce an increased risk of large bowel cancer (Palmer and Spratt, 1956; Court Brown and Doll, 1965).

It is plausible that pelvic X-irradiation should induce rectal carcinoma in man since it does so in mice (Hirose et al, 1977). Whole-body irradiation can also induce carcinomas of the large and small bowel in both rats and mice (Brecher et al, 1953; Casarett, 1965), though fast neutrons may be more effective (Nowell et al, 1956; Nowell and Cole, 1959; Upton et al, 1960). Intragastric administration of $^{91}\text{yttrium}$ or $^{144}\text{cerium}$ produces intestinal tumours (Lisco et al, 1947; Lebedeva, 1964). Osborne and colleagues have developed the best model of intestinal radiocarcinogenesis in which segments of small or large bowel are rendered temporarily hypoxic by vascular clamping, allowing the delivery of high doses of X-irradiation (up to 6500 R) to the intestine (Osborne et al, 1963; Coop et al, 1974; Denman et al, 1978). The number of neoplasms induced is dose-related. At high doses problems arise with toxicity, chronic inflammation and stricturing. Radiation-induced tumours resemble both human cancers and experimental tumours induced by chemical carcinogens.

Suture-line "recurrence"

In 1907, Sir Charles Ryall proposed that anastomotic recurrence

following potentially curative resection of colonic carcinoma arose from implantation of malignant cells at the time of surgery. Subsequently many surgeons have employed various measures to prevent such local implantation: early colonic ligation (Cole et al, 1954), or instillation of cytotoxic agents into the bowel ends before anastomosis (McDonald et al, 1960; Gibson and Stephens, 1966).

Studies in rabbits suggest that intraluminal antiseptics favours the development of suture-line cancer (Vink, 1954; Cohn, 1967), yet iodised catgut sutures have an inhibitory effect on both implantation metastasis and suture-transferral of malignant cells (Herter and Sbuelz, 1966; Keller et al, 1966; Cohn, 1967).

In man, occasional reports of metastases in raw areas adjacent to the resection site support the concept of malignant implantation in clinical practice (Goligher, 1984). Suture-line "recurrence" occurs in 6-36% of patients undergoing radical resection for large bowel cancer (Cohn, 1967). Many of these develop after such a long latent period that it seems unlikely that they arose from cancer cells seeded at the original operation. However, a proportion of early suture-line "recurrences" may be due to inadequate primary resection or retrograde lymphatic spread.

Malignant cells can be detected both in the bowel lumen and in the bloodstream at operation but their viability is uncertain. Rosenberg (1979) found that exfoliated cells from human and experimental cancers were non-viable in that they failed to exclude trypan blue or take up thymidine. By contrast, Umpleby et al (1984) have found that many such cells do exclude trypan blue and may therefore be potentially viable and capable of implantation.

Suture-line cancers could arise de novo as a result of chronic foreign body irritation. Pozharisski (1975) inserted a silk stitch into the caecum of

rats receiving dimethylhydrazine producing a focus of chronic inflammation in the caecal wall which was the site of a large number of carcinomas.

Ureterosigmoidostomy

The ureterocolic anastomosis is an interesting site for the development of large bowel cancer. The first case was described by Hammer in 1929, since when over 50 cases have been reported. In the majority urinary diversion had been carried out for benign conditions, notably exstrophy (Shapiro et al, 1979; Spence et al, 1979). The latent interval ranges from 6-50 years and is markedly shorter after ureterosigmoidostomy for malignant disease (Parsons et al, 1977; Leadbetter et al, 1979; Shapiro et al, 1979; Spence et al, 1979). The commonest histological type is adenocarcinoma, but adenomas and even transitional cell carcinomas have been found (Whitaker et al, 1971; Spence et al, 1979). The risk of colonic cancer in an individual with ureterosigmoidostomy is said to be 100-550 times normal (Urdaneta et al, 1966; Parsons et al, 1977; Sooriyaarachchi et al, 1977). In a recent prospective study of 34 patients with longstanding ureterosigmoidostomy, Stewart and Hill (1983) found that 29% had developed either anastomotic tumour or field change (dysplasia).

The mechanism of carcinogenesis in this model remains uncertain. Direct action of urine on bowel mucosa seems unlikely as there have been five cases to date in which tumours arose at a ureteric anastomosis which had long been defunctioned by nephrectomy or conversion to an ileal loop conduit (Spence et al, 1979). Moreover tumours are not distributed throughout the bowel exposed to urine but are confined to the anastomosis (Whitaker et al, 1971; Parsons et al, 1977).

Experimental animal studies on ureterosigmoidostomy have produced

conflicting results: dogs given one urinary carcinogen (beta-naphthylamine) failed to develop bowel cancers (Scott and Boyd, 1953), whereas rats given either a different urinary carcinogen (N-(4-(5-nitro-2-furyl)2-thiazolyl)formamide) or an intestinal carcinogen (dimethylhydrazine) did develop anastomotic tumours but not if the distal colon was defunctioned by a proximal colostomy (Crissey et al, 1980; Steele et al, 1981).

Chronic faecal irritation of the ureteric mucosa seems unlikely as histological appearances suggest an origin from the colonic mucosa (Parsons et al, 1977). Furthermore carcinoma has not yet been reported in an ileal urinary conduit. Mechanical trauma or chronic inflammation of the colonic mucosa around the anastomosis seem the likeliest aetiological factors (Bristol and Williamson, 1981). Recurrent trauma probably explains the development of carcinomas of the rectum in mice with chronic rectal prolapse (Wells et al, 1938) and cloacogenic cancer of the anorectum in male homosexuals engaging in longstanding receptive anal intercourse (Cooper et al, 1979).

CHAPTER 5
INTESTINAL CELL PROLIFERATION

Normal mucosal cell tumour

The small bowel and colonic epithelia are in a constant state of renewal. Intestinal mucosal turnover is the fastest for any tissue in the body and is exceeded only by a few rapidly growing malignancies (Williamson, 1978). Cell renewal follows a similar general pattern in both small and large bowel, although events are marginally slower and less uniform in the latter. Undifferentiated stem cells at the base of the crypts give rise to three main cell types (Chang and Leblond, 1971; Cheng and Leblond, 1974). The commonest of these, the "chief", or columnar cell arises in the small bowel by mitotic division within the crypt and migrates onto the villus, where it differentiates into the mature form capable of absorbing nutrients but having lost the ability to synthesise DNA and divide (Imondi et al, 1969; Lipkin, 1973). In the colonic crypt, cells differentiate as they migrate upwards and are extruded onto the mucosal surface as mature cells. In normal mucosa proliferating cells are confined to the lower two-thirds of the crypts. Cells are discarded into the bowel lumen from the villus tip or from the colonic mucosal surface. Under normal steady-state conditions in the absence of inflammation cell loss into the lumen is balanced by cell birth at the crypt base (Williamson, 1978).

Goblet (mucous) cells also differentiate from basal stem cells or may arise by proliferation of oligomucous cells in the crypt (Cheng and Leblond, 1974; Cheng, 1974). They migrate upwards differentiating into two subtypes: common and granular mucous cells. Endocrine (argentaffin) cells probably differentiate from stem cells (Cheng and Leblond, 1974) and migrate up to the villus or mucosal surface with a slightly longer turnover time in the mouse (4 days) than columnar and mucous cells (3 days) (Cheng and Leblond, 1974). Stem cells may also give rise to a fourth cell type, the

Paneth cell, which is normally confined to the small bowel mucosa and does not migrate. These cells remain in the crypt base and ultimately degenerate and undergo phagocytosis (Cheng, 1974).

The small bowel epithelium is completely replaced within 2-3 days in mice and rats and within 3-6 days in man. Turnover is slower in the colon, taking 4-8 days in man (Williamson, 1978). The mucosal crypts are surrounded by a sheath of fibroblasts within the lamina propria (Pascal et al, 1968; Parker et al, 1974). These cells also undergo rapid renewal which may be intimately coordinated with mucosal cell renewal in the rabbit (Pascal et al, 1968), if not in the rat (Maskens et al, 1979).

Intestinal cell kinetics are best studied by autoradiography or by stathmokinetic techniques. Tritiated thymidine ($^3\text{HTdR}$) is a labelled nucleoside precursor, incorporated exclusively into DNA, which when injected into animals forms the basis of autoradiographic determination of DNA synthesis and cell proliferation. The stathmokinetic technique employs agents such as colchicine and vincristine which halt cells in metaphase. Counting arrested metaphases in crypts at different times after injection of the agent enables calculation of the crypt cell production rate (Al-Mukhtar et al, 1982) (Chapter 9).

The cell cycle time varies between 10-24 hours depending on the species (Williamson, 1978). The S phase (DNA synthesis) takes up about half this time during which $^3\text{HTdR}$ is incorporated (Lipkin et al, 1963). Protein and RNA synthesis continue through the succeeding premitotic gap (G₂) and the preceding postmitotic gap (G₁) (Eastwood, 1977). Mitosis itself lasts barely an hour. In the colon these phases are slightly longer and a few cells enter the prolonged interphase (G₀) found in more slowly-renewing tissues (Lipkin, 1973; Williamson, 1978).

One to six percent of $^3\text{HTdR}$ -labelled cells may be detected in both gastric and colonic epithelium one week after injection, compared to 0.3% remaining in the small bowel (Lipkin and Quastler, 1962). Such prolonged retention of cells in the mucosa, where they may be exposed to mutagenic and carcinogenic factors, might partly account for the relatively greater susceptibility to cancer of the colon and the stomach compared to the small bowel.

Pre-neoplastic cell kinetics

The initial reaction of the gut mucosa to administration of a cyasin analogue is an inhibition of DNA and RNA synthesis evident within one hour (Zedeck et al, 1970). Prominent necrosis of crypt cells in both large and small bowel mucosae is extensive within six hours (Zedeck et al, 1970; Sunter, 1980, Sunter et al, 1981). Cell damage, evidenced by nuclear pyknosis and karyorrhexis, is confined to the region of the crypt where actively proliferating cells are found. The distribution of acute mucosal damage appears to parallel the distribution of tumours, which ultimately arise with continued carcinogen administration (Zedeck et al, 1977). In rodents this severe cytotoxic effect lasts about 48 hours and is accompanied by a transient depression of crypt cell production (Zedeck, 1978; Lamont and O'Gorman, 1978). This phase is followed by a prominent compensatory proliferative response (Sunter et al, 1981). There are increases in both labelling and mitotic indices at 36-48 hours and actively-dividing, DNA-synthesising cells appear high in the crypt. This crypt hyperplasia is evident by about 4 days but is rapidly curtailed, so that by 7 days the kinetic state appears normal again (Wright, 1983).

This recovery is succeeded by a long latent period during which a further gradual increase in cell proliferation can be detected in mice as

early as 2-5 weeks after starting treatment (Thurnherr et al, 1973; Richards, 1977). Following DMH administration the main autoradiographic finding is an expansion of the proliferative compartment of the colonic crypt in rats and mice (Lohrs et al, 1969; Springer et al, 1970; Tutton and Barkla, 1976; Thurnherr et al, 1973; Wiebecke et al, 1973; Deschner, 1974; Chang et al, 1979). There is an associated increase of crypt length and girth proportional to the duration of carcinogen treatment (Chang, 1978, 1980), but there is no disturbance of cellular differentiation. Both the labelling index and the total number of crypt cells are increased. There is an upward extension of the proliferative compartment and actively dividing cells appear in the upper third of the crypt, normally the preserve of fully mature cells. This is accompanied by an overall increase in the number of proliferating cells (Maskens, 1976; Richards, 1977; Sunter et al, 1981).

Changes in the individual components of the cell cycle time are less well established. The S phase is probably unchanged (Richards, 1977; Sunter et al, 1981), but both shortening and prolongation of the G1 phase and overall cell cycle time have been reported (Chang et al, 1979; Pozharisski et al, 1979; Sunter et al, 1981).

These hyperplastic changes might simply reflect acute epithelial damage (Chan et al, 1976; Richards, 1977), and it is not known whether they differ from the hyperplasia seen in hyperplastic (metaplastic) colonic polyps found in man which are not considered to be premalignant (Lane et al, 1971; Hayashi et al, 1974).

Lipkin and Deschner have defined two phases of preneoplastic change in premalignant colorectal mucosa in rodents and humans (Deschner et al, 1963; Lipkin, 1974a,b; Deschner and Lipkin, 1975; Deschner, 1982). In phase 1 lesions there is normal cell turnover but there is failure of the

normal repression of thymidine uptake that accompanies cell migration towards the surface. These abnormal cells accumulate in the upper crypt forming aberrant and uncontrolled proliferative foci: phase 2 lesions (Deschner, 1982). These changes have been observed in the mucosa of patients with familial polyposis, their asymptomatic relatives, and in patients with isolated adenomas and colorectal cancer (Deschner, 1982). Such similar kinetic changes in what is generally considered to be premalignant human mucosa and in the mucosa of carcinogen-treated rodents suggest a common process of malignant transformation and raise the possibility of screening for malignant change in high-risk individuals (Lipkin, 1975; Deschner, 1982).

Neoplastic cell kinetics

The study of the cell kinetics of both spontaneous human colorectal cancers and chemically induced cancers in rodents is important for understanding the nature of the disease and for designing rational cytotoxic therapy (Chang et al, 1979). Unfortunately the kinetic properties of colorectal tumours have been relatively poorly defined, perhaps because there is great variability between individual neoplasms. In one study of six benign adenomas, for example, the $^3\text{HTdR}$ labelling index was 3-11 times lower in surface cells than in those 0.1 mm below the surface (Sunter, 1980). Human cancers also vary widely in their kinetic behaviour. Estimates of individual doubling time range from 2 days to 3 years (Bottomley and Cooper, 1973; Leshner et al, 1977). Autoradiographic and stathmokinetic studies of normal and malignant human colonic tissues in vivo and in vitro have generally indicated that carcinoma cells remain longer in S phase and

thus have a longer cell cycle time than normal cells. Hence cancer cells appear to proliferate more slowly than normal which may partly explain the relative resistance of colorectal cancer to cytotoxic therapy (Hoffman and Post, 1967; Bottomley and Cooper, 1973; Camplejohn et al, 1973; Bleiberg et al, 1977). However in one study about 10-20% of large bowel carcinomas displayed accelerated proliferative activity, and a similar percentage is recorded for the response of bowel cancers to cytotoxic therapy (Falkson and Falkson, 1976; Leshner et al, 1977). A similar though less marked trend has been observed in DMH-induced colorectal neoplasms in rats (Tutton and Barkla, 1976). By contrast, the cell cycle time may be decreased compared to normal in neoplastic colonic cells in mice (Deschner and Lipkin, 1975) or effectively unchanged despite variations in duration of individual components of the cycle (Tutton and Barkla, 1978; Chang et al, 1979). The growth fraction may be increased (Chang et al, 1979) or decreased (Sunter et al, 1980).

A polyp is characterised as neoplastic rather than hyperplastic if proliferating cells (actively incorporating $^3\text{HTdR}$) are found on its surface (Hayashi et al, 1974; Deschner and Lipkin, 1975). The enzymatic properties of surface cells also differ in neoplastic and hyperplastic lesions (Lipkin, 1974a,b; Pozharriski et al, 1979). Superficial cells of adenomas and carcinomas have high levels of thymidine kinase activity, usually found in actively dividing cells, but they have reduced levels of enzymes associated with cell differentiation. Proliferative disorders of the underlying pericrypt or sheath are seen during colonic neoplasia but not in hyperplastic lesions (Kaye et al, 1971).

CHAPTER 6
SMALL BOWEL ADAPTATION

Intestinal Adaptation

The normal rapid proliferative state of the enterocolic mucosa in animals and man can be accelerated or decelerated by a number of stimuli. Age, diurnal rhythm, alteration in nutrient intake, neurovascular and endocrine changes, and mucosal injury all modulate the rate of crypt cell proliferation (Leblond and Walker, 1956; Steiner et al, 1968; Williamson, 1978). The most marked changes are produced by surgical manipulations. Partial small bowel resection in particular produces a marked increase in the rate of cell proliferation in the remaining gut which has been extensively studied in both animal and man. This appears to be a compensatory response enabling the organism to adapt to the loss of functioning intestine.

In man, survival is possible after loss of most of the small bowel and postoperative diarrhoea and malabsorption tend to improve with time. The gut, like the liver and the kidney, has both a functional reserve and the capacity to regenerate (Williamson, 1978).

In 1888 Senn of Milwaukee noted that, in dogs and cats surviving partial small bowel resection, the residual small bowel was "much thickened and exceedingly vascular". This finding, which he termed "compensatory hypertrophy", was confirmed by Monari in 1896. Flint (1912) resected varying amounts of small bowel in dogs and found a striking increase in the transverse dimension of the remaining bowel. Its length was unaffected, which he attributed to fixation to the inelastic mesentery. All intestinal layers were involved in this thickening, especially the mucosa and circular muscle. He also found that villi were increased in size but not in number resulting in an overall compensatory increase in absorptive surface area. He cautioned that this capacity of residual bowel to compensate might be

overwhelmed by too extensive a resection. Most subsequent studies confirmed these findings but several workers failed to detect any hypertrophy in residual bowel in animals and man (Trzebicky, 1894; Wildegans, 1925). The 1.05 m jejunal remnant in a young woman surviving massive (4 m) small bowel resection was examined by Butler (1959) at her four subsequent Caesarian sections. He found no increase in length, diameter or thickness and concluded that as she had made a rapid and complete recovery following resection, the small bowel held a considerable functional reserve. Flint (1912) observed large numbers of mitotic figures in bowel remnants and proposed that the process of structural adaptation involved hyperplasia as distinct from the hypertrophy suggested by Senn. This hypothesis has only been confirmed by cellular studies carried out during the last 20-25 years.

The first successful recorded massive intestinal resection in man (2.05 m) has been attributed to Koeberle (1881) in Strasbourg, and in the succeeding 100 years surgeons have attempted to define the upper limit of small bowel resection compatible with long-term survival. "Massive" resections (greater than 2 m, thought to be about one third of the small bowel) were carried out for volvulus, strangulated hernia, mesenteric vascular disease, intestinal tuberculosis, trauma and adhesions. Flint (1912) collected a series of 58 such cases from the world literature and reported an overall mortality of 16%, which he thought to be a considerable underestimate because of surgeons' natural reluctance to publish unsuccessful cases.

The normal total length of the small bowel is extremely variable (Bryant, 1924) and measurements and estimates of the length of excised segments notoriously inaccurate. Consequently reported estimates of the

extent of resections based on the length of the excised specimen are difficult to interpret and surgeons have realised that the length of the residual bowel is a more satisfactory index. However, over the years a consensus gradually emerged from clinical and experimental work: one third of the small bowel could be excised without significant effect, one half was the upper limit of safe resection and removal of three quarters or more might occasionally be survived but never adequately compensated. More recently, however, several cases of survival have been reported in patients with as little as 6-18 inches of residual small bowel (Linder et al, 1953; Anderson, 1965; Winawer and Zamcheck, 1968). One patient survived for at least a year after removal of the entire jejunum, ileum and right colon for midgut volvulus (Kinney et al, 1962).

The study of intestinal adaptation to loss of functioning bowel has become increasingly relevant in clinical practice with development of intravenous hyperalimentation leading to the survival of patients with massive resections who would otherwise have died from shortage of functioning bowel. Moreover, large numbers of massive jejunoileal bypass operations are now carried out in the management of obesity in relatively young and otherwise healthy individuals.

Much more is known about the adaptive response of the small bowel to various stimuli (which is reviewed in this chapter) than about colonic adaptation (discussed in Chapter 7). Most current knowledge has been gleaned from experiments involving laboratory animals, particularly rats and mice.

Small Bowel Adaptation

Resection of a major portion of the small bowel produces adaptive

changes affecting all layers of the remaining small bowel, resulting in dilatation, thickening and lengthening of the gut together with hyperplasia of the crypt/villus system (Nygaard, 1967; Wilmore et al, 1971). Villus height and crypt depth are increased associated with an overall increase in their cell populations (Dowling and Booth, 1967; Nygaard, 1967; Hanson and Osborne, 1971; Hanson et al, 1977; Williamson et al, 1977). However, the number of cells per unit length of villus and mucosal RNA/DNA ratios are unchanged indicating that mucosal proliferation is achieved by hyperplasia rather than hypertrophy (Hanson et al, 1977; Williamson et al, 1978a). The degree of these changes is related to the extent of resection (Hanson et al, 1977; Williamson et al, 1978a). It is always greater after proximal than after distal resection and maximal immediately distal to the anastomosis (Booth et al, 1959; Dowling and Booth, 1967).

Morphometrically detectable adaptation reaches a peak 7-12 days after enterectomy (Williamson, 1978), but measurements of mucosal RNA, DNA and $^3\text{HTdR}$ -labelled radioactivity reveal intense hyperplasia within 48 hours of operation (Obertop et al, 1977; Oscarson et al, 1977; Williamson et al, 1978a).

Both the number of proliferating cells and the total number of cells in the crypt column are increased, so the labelling index remains approximately constant (Hanson and Osborne, 1971; Hanson et al, 1977). The S or G₁ phases of the cell cycle are shortened producing a slight overall reduction in the cell cycle time (Hanson and Osborne, 1971; McDermott and Roudnew, 1976). Cells migrate more rapidly along the elongated crypt and villus, but after an initial increase cell turnover rate appears essentially unchanged (Dowling and Gleeson, 1973).

Initial studies suggested that intestinal absorption of substances such

as water, monosaccharides, disaccharides, amino acids and calcium was increased following resection (Dowling and Booth, 1967; Bury, 1972; Urban and Pena, 1974). However, when expressed in terms of mucosal weight or protein content, absorption of these substances is unchanged (Dowling, 1974). Furthermore, mucosal disaccharidase and dipeptidase activities are normal or decreased after resection suggesting a comparative immaturity of cells in the hyperplastic mucosa (Weser and Hernandez, 1971; Bury, 1972). Functional adaptation, therefore, appears to depend on increased numbers of epithelial cells which do not have individually enhanced absorptive capacity (Dowling, 1974).

In man, functional adaptation is evidenced by the gradual improvement of diarrhoea and malabsorption which follows survival from massive intestinal resection (Williamson, 1978).

Small bowel **bypass**, by exclusion as a self emptying blind loop anastomosed end-to-side to the ileum or colon, or as a Thiry-Vella fistula draining to the surface, produces adaptive changes similar to those found after resection (Nygaard, 1967; Gleeson et al, 1972; Menge et al, 1974). The initial response to bypass is slower than that following resection of an equivalent segment (Williamson et al, 1978a), but by four weeks there is no detectable difference in the hyperplasia produced by the two procedures (Nygaard, 1967; Gleeson et al, 1972; Williamson et al, 1978a). Functional adaptation is much less effective after bypass than after resection probably because of bacterial colonisation of the bypassed loop (Nygaard, 1968).

In the bypassed loop itself marked hypoplasia develops within 1-2 weeks of isolation (Rijke et al, 1977). Cell production and crypt/villus cell populations are decreased, migration is slowed and cell turnover is decreased (Gleeson et al, 1972; Menge et al, 1974; Rijke et al, 1977;

Williamson et al, 1978a). Mucosal wet weight and nucleic acid content are reduced. Villous height and crypt depth may be decreased or unaltered, the lumen is narrowed and the bowel wall becomes progressively thinner (Fenyo and Hallberg, 1976). Functional atrophy is revealed by decreased segmental absorption of glucose and amino acids (Gleeson et al, 1972). These changes are all rapidly reversed by restoration of intestinal continuity (Keren et al, 1975; Fenyo and Hallberg, 1976).

In man there is adaptive hyperplasia and increased segmental absorption of nutrients in bowel remaining in continuity after jejunoileal bypass carried out in the management of morbid obesity (Iversen et al, 1976). At subsequent laparotomy the bypassed loop is thin-walled and narrow compared with the thickened elongated bowel in continuity (Williamson, 1978).

Simple **transection and reanastomosis** alone causes transient hyperplasia immediately proximal to the anastomosis (Rijke et al, 1977) or throughout the distal small bowel (Williamson et al, 1978b; Williamson and Malt, 1980). **Subtotal colectomy** produces ileal hyperplasia which develops more slowly than that after jejunectomy (Bucholtz et al, 1976; Wright et al, 1969). The mucosa of **ileal segments transposed** to the jejunum quickly develops increased morphometric dimensions and comes to resemble the adjacent jejunal mucosa (Dowling and Booth, 1967; Altmann and Leblond, 1970; Rijke et al, 1977) but the effect of transposing jejunal segments to the ileum is uncertain (Williamson, 1978). **Diversion of bile and pancreatic secretions** to mid small bowel produces ileal hyperplasia and mild transient hypoplasia of the jejunum (Williamson et al, 1978c, Chapter 8).

Other modulators of enteric proliferation

Withdrawal of oral feeding by total **starvation** with or without

parenteral nutrition produces hypoplasia throughout the enteric mucosa (Steiner et al, 1968; Levin et al, 1974; Johnson et al, 1975a), which is reversed by refeeding (Altmann, 1972; Al Dewachi et al, 1975). **Reduced nutrient intake** in semistarvation, malignant cachexia or bulk dilution of the diet all produce enhanced absorptive capacity in individual epithelial cells without increasing their number (Wiseman et al, 1959; Kershaw et al, 1960; Dowling, 1967; Dowling et al, 1967). In contrast to the large bowel, physical stimulation by **distension** or increased dietary **bulk** does not produce hyperplasia in the small bowel (Dowling et al, 1967). Lactation and experimental models of hyper^hpagia induced by hypothalamic damage, hyperthyroidism, hypothermia and diabetes mellitus can all produce generalised enteric hyperplasia (Williamson, 1978).

Mucosal damage caused by irradiation or cytotoxic agents results in decreased cell production followed by enhanced proliferation leading to recovery (Trier and Browning, 1966; Rijke et al, 1975). **Folate** and **B₁₂ deficiency** in man both lead to villous shortening and decreased mitotic activity reversed by appropriate replacement therapy (Foroozan and Trier, 1967; Hermos et al, 1972). The **inflammation** of **infective enteritis** (bacterial, viral or parasitic) and **coeliac disease** produces villous shortening but increased crypt cell proliferation (Symons, 1965; Schrieber et al, 1973; Zufarov et al, 1973). **Bacteria** are important in the maintenance of normal enteric epithelial cell turnover which is depressed in germ-free mice (Abrams et al, 1962; Leshner et al, 1964).

The control of small intestinal adaptation

Proliferation of the small gut epithelium may be regulated by a negative feedback system whereby cell division in the progenitor

compartment (crypt) is controlled by the number of cells in the functional compartment (villus) (Williamson, 1978).

In addition the adaptive response of small bowel epithelium to various stimuli appears to be mediated by a number of extrinsic mechanisms the most important of which are intraluminal and humoral (or systemic) factors. The gut mucosa is ideally sited for exposure to substances both in the lumen and delivered via the bloodstream.

The theory of luminal or topical nutrition was proposed and elaborated by Dowling and co-workers (Dowling and Booth, 1967; Gleeson et al, 1972; Dowling and Gleeson, 1973). Jejunal mucosa is normally exposed to high concentrations of nutrients; exogenous carbohydrate, fat and protein, which are normally almost completely absorbed before the chyme reaches the ileum. After proximal enterectomy, these nutrients would reach the ileum in much higher than normal concentrations and might directly stimulate hyperplasia. However, this mechanism is unlikely to account for the parallel adaptive changes in the muscle coat (Nygaard, 1974). The normal aboral gradient of villus height decreasing from pylorus to ileocaecal valve (Altmann and Enesco, 1967; Levine et al, 1974), and the greater adaptive effect of proximal compared to distal small bowel resection (Dowling and Booth, 1967) could both be explained by topical nutrition. The hyperplasia of ileal segments transposed to the jejunum partly confirms this theory (Dowling and Booth, 1967; Altmann and Leblond, 1970; Rijke et al, 1977). In this model the mucosa is subjected to a change in luminal environment in the absence of resection. Conversely removal of luminal nutrition by starvation or surgical exclusion leads to progressive structural and functional atrophy (Gleeson et al, 1972; Rijke et al, 1977). The jejunum is more sensitive to this effect than the ileum probably because it depends on

the higher nutrient concentrations to which it is usually exposed (Williamson and Malt, 1980). Moreover, total parenteral nutrition abolishes the normal adaptive effects of proximal enterectomy in rats and dogs (Feldman et al, 1976; Levine et al, 1976) and reduces but does not abolish adaptation after subtotal enteric bypass in rats (Fenyo et al, 1976). Perfusion of isolated small bowel Thiry-Vella fistulas with glucose or amino acids prevents the hypoplasia normally found in these loops (Altmann, 1974; Jacobs et al, 1975; Menge et al, 1975).

Food in the gut might act indirectly by stimulating the release of endogenous factors such as gastric, duodenal and pancreaticobiliary secretions. Jejunal resection and bypass bring the ileum closer to the sources of these substances, exposing it to higher than normal levels. Both gastric and duodenal secretions increase ileal villous height but pancreaticobiliary secretions exert a stronger effect (Altmann and Leblond, 1970; Altmann, 1971). Diversion of the duodenal papilla to mid small bowel causes rapid and lasting ileal hyperplasia (Williamson et al, 1978c) and enhances ileal adaptation following jejunectomy (Weser et al, 1977). The effect of the combined pancreaticobiliary juice is greater than that of bile alone (Williamson et al, 1978c).

Infusion of pancreatic extracts into isolated loops of small bowel produces greater hyperplasia than infusion of amino acids alone (Altmann, 1974) suggesting that pancreaticobiliary juice does not exert its tropic effect merely by increasing the availability of amino acids to the mucosa. This effect is more likely to be related either to alterations in mucosal permeability or to chronic irritation and inflammation (Williamson et al, 1978c).

The presence of food in the gut may be necessary for the release of

endogenous secretions and for them to exert their effects. Starvation or parenteral nutrition may induce mucosal atrophy partly by reducing the release of these substances (Williamson, 1978).

The theory of luminal nutrition cannot explain all aspects of intestinal adaptation and there is a great deal of circumstantial evidence implicating humoral factors in the control of the adaptive response. Simple transection and reanastomosis of the jejunum produces transient hyperplasia of the distal ileum which is more readily attributable to systemic influence than to any alteration in luminal contents (Williamson and Malt, 1980). The rapidity and uniformity of the enteric response to the various surgical stimuli discussed above may well reflect a systemic control mechanism.

Intact pigs and rats linked by vascular parabiosis to partners undergoing intestinal operations show increased uptake of thymidine in their small bowel mucosa; a finding which could be explained by the systemic release of a stimulatory hormone (Laplace, 1973; Williamson et al, 1978b). A similar mechanism might also explain the rapid hyperplasia following jejunectomy both in the duodenum proximal to the site of resection, and to a lesser extent in upper ileum isolated as a Thiry-Vella fistula (Hanson et al, 1977; Williamson and Malt, 1980). Compensatory hyperplasia in the ileum after colectomy (Wright et al, 1969; Bucholtz et al, 1976) and in mucosal autografts (Tilson and Livstone, 1975) may also depend on a systemic stimulatory mechanism.

Mucosal growth may also be regulated by an inhibitory hormone. Bypass procedures produce a much less intense adaptive response in the first postoperative week than resection. By about one month the effects of resection and the equivalent bypass procedure are similar (Williamson et al, 1978a). The level of an inhibitory factor present in the mucosa could be

reduced immediately after resection but would diminish more gradually after bypass as the defunctioned segment atrophied. Mucosal regeneration restoring original levels could thus proceed more rapidly after resection than after bypass (Tilson and Wright 1970; Williamson and Malt, 1980).

Hormones also seem likely to be among controlling elements in adaptive intestinal growth during lactation, diabetes, and hypothermia (Williamson, 1978).

Many substances have been suggested as possible enterotropic hormones but two gastrointestinal peptides, **gastrin** and **enteroglucagon** have emerged as the most likely candidates. **Gastrin** is tropic to both the stomach and duodenum (Crean et al, 1974; Johnson et al, 1975b). Evidence supporting a tropic role for gastrin distal to the duodenum is equivocal. A single injection of pentagastrin increases thymidine uptake in ileal and colonic crypts (Panson et al, 1974; Mak and Chang, 1976) and chronic endogenous hypergastrinaemia increases intestinal weight (MacGregor and Way, 1976). By contrast, other studies have not confirmed such an effect (Mayston et al, 1975; Morin and Ling, 1978). Moreover, 20-fold variations of endogenous serum gastrin produced by different gastric operations neither prevent the enteric hypoplasia of starvation nor affect post-resectional hyperplasia (Oscarson et al, 1977).

Enteroglucagon was first implicated as a possible tropic gut hormone following a report of a patient with an enteroglucagon-producing renal tumour who exhibited marked hyperplasia of all coats of the intestine (Gleeson et al, 1971). Following excision of the tumour the appearance of the intestine reverted to normal. Plasma enteroglucagon is raised postprandially in patients with untreated coeliac disease (Besterman et al, 1978a), after small bowel resection (Bloom et al, 1979) and jejunoileal

bypass (Besterman et al, 1978b; Kulneff-Herlin et al, 1982): conditions in which there is malabsorption and compensatory hyperplasia in residual gut-in-continuity. In rats with 75% of the small bowel isolated as a Thiry-Vella fistula, plasma enteroglucagon is raised and there is increased crypt cell production rate both in residual bowel-in-continuity and in the fistula itself (Sagar et al, 1983). These effects are reduced if the animals are fed intravenously, suggesting that both enteroglucagon release and mucosal hyperplasia are influenced by luminal nutrition.

In man, enteroglucagon-secreting cells occur in the highest numbers in the distal ileum and also in the colon (Bloom, 1980). Sagar and co-workers (1982) have suggested that enteroglucagon is normally released rapidly after a meal. In malabsorption following extensive small bowel resection or bypass, the distal ileum is exposed to chyme abnormally rich in unabsorbed nutrients. Enteroglucagon is thus released in excessive quantities which has the effect of increasing intestinal transit time and stimulating cellular proliferation, thus enhancing absorption.

Other hormones, **mineralocorticoids** (Tilson et al, 1971), **glucocorticoids** (Tutton, 1973), **pituitary hormones** (Taylor et al, 1975), **testosterone** (Wright et al, 1972) and **thyroxine** (Leblond and Carriere, 1955) may stimulate enteric proliferation. **Serotonin** and **histamine** stimulate proliferation in small doses, whereas large doses are inhibitory (Tutton, 1977). **Cholecystokinin** is weakly tropic to the duodenum (Johnson and Guthrie, 1976). **Secretin** inhibits gastrin release and is antitropic to rat jejunum, an effect possibly shared by **vasoactive intestinal peptide** (Stanley et al, 1972; Enochs and Johnson, 1977).

While humoral agents have an important role in small intestinal

adaptation the direct effect of food in the gut lumen remains the dominant influence. The response in functioning loops exceeds that in loops isolated from bowel content, distal adaptation exceeds proximal adaptation and hyperplasia is always greater in parabionts actually undergoing intestinal surgery than in their intact partners. Luminal content may exert its effect directly or indirectly by stimulating the release of endogenous and luminal factors and consequently adaptation is maximal when topical and systemic factors coincide.

CHAPTER 7

ADAPTATION AND CARCINOGENESIS IN THE LARGE BOWEL

In man, the large bowel makes up a relatively minor proportion of the total intestinal length and surface area yet carcinoma develops one hundred times more frequently in the colorectum than in the jejunioileum (Brookes et al, 1968; Reyes and Talley, 1970). Inflammatory conditions are also more prevalent in the large bowel. It seems inappropriate, therefore, that while epithelial cell proliferation and adaptation have been extensively investigated in the small gut, comparatively little is known about these processes in the colon. The main function of the large gut appears to lie in the reabsorption of fluid rather than in digestion and absorption of nutrients. There are certain basic anatomical differences between the large and small intestines, notably the incomplete longitudinal muscle layer and the absence of villi in the colorectum. It is possible that subtle differences in epithelial cytokinetics between the two regions might explain the greater potential of the large bowel mucosa for neoplastic change (Bristol and Williamson, 1984).

Microscopically, individual crypts with their associated cuffs of mucosal surface probably represent units of proliferative and functional activity (Al-Mukhtar et al, 1982). As in the small bowel, there is a small pool of slowly cycling stem cells situated in the base of colonic crypts (Potton et al, 1979). Above this level a zone of actively proliferating cells extends up to a point almost two-thirds of the distance from base to crypt orifice (Williamson, 1978). In the upper third of the crypt, cells no longer capable of dividing differentiate to functional maturity before they reach the mucosal surface from which they are subsequently extruded into the lumen. Basal stem cells give rise to three main cell types: columnar, mucous and endocrine (Chang and Leblond, 1971). Cell division and migration take slightly longer in the large bowel than in enteric mucosa and turnover time of the colonic mucosa has been estimated at 4 to 8 days in

man (Lipkin and Deschner, 1976) and 2 to 5 days in rodents (Chang and Leblond, 1971; Obertop et al, 1977).

Colorectal adaptation

Compensatory hyperplasia of the colon and rectum to various stimuli is less easy to detect than in the small bowel but in general it seems to be a less striking and comparatively transient phenomenon (Bristol and Williamson, 1984). Although varying degrees of **colonic resection** consistently produce adaptive hyperplasia of the residual small gut (Chapter 6), their effect on the remaining large bowel is less impressive. Increases have been found in mucosal wet weight, protein and nucleic acid contents of the right colon 40 weeks after left hemicolectomy or caecectomy (Williamson et al, 1982). By contrast, the left colon does not appear to respond to right hemicolectomy or caecectomy. When caecectomy is combined with resection of the distal 50 cm of ileum, modest increases in mucosal thickness are seen throughout the colon (Scarpello et al, 1978). The rectum is capable of adapting to subtotal colectomy and ileorectal anastomosis (Owen and Lyttle, 1979).

Diversion of the faecal stream away from the distal colonic mucosa by proximal **defunctioning colostomy** leads to profound mucosal hypoplasia which is rapidly and precisely reversed when normal intestinal continuity is restored (Rijke et al, 1979; Terpstra et al, 1981).

Ileal resection produces intense hyperplasia throughout the large bowel demonstrable within 2 weeks by increased mucosal nucleic acid levels, and ³HTdR-labelled radioactivity (Tilson et al, 1976; Nundy et al, 1977; Williamson et al, 1978a). These changes may occur within 48 hours but while small bowel hyperplasia has been detected up to 3 months after

enterectomy, colonic hyperplasia has almost completely subsided (Williamson et al, 1977), although caecal adaptation may last longer (Scarpello et al, 1978; Williamson et al, 1980a). Whereas limited **enteric bypass** (50% proximal small bowel) does not seem to affect colonic mucosal mass (Williamson et al, 1978a), subtotal (85%) jejunoileal bypass produces sustained hyperplasia with increased length, weight, crypt depth and crypt cell production rate throughout the large intestine at 30 weeks (Bristol et al, 1984). **Pancreaticobiliary diversion** to mid-small bowel has little effect on the colonic mucosa compared to the appreciable and persistent growth it produces in ileal mucosa (Weser et al, 1977; Williamson et al, 1978).

Functional adaptation in the colon is poorly documented. One recent study demonstrated an increase in colonic absorption of sodium chloride and water following 70% small bowel resection which was proportional to the increase in colonic mucosal mass. This functional adaptation is similar to that in the small bowel in that it appears to result from an increase in cell number rather than increased absorptive capacity of individual cells (Urban et al, 1983). Interestingly the large bowel of newborn rats can absorb sugar, an ability which is absent in mature rats and does not reappear even after major small bowel resection (Urban et al, 1983).

In man, the colon may increase its capacity to absorb water and electrolytes and adapt to absorb glucose and amino acids after **jejunoileal** bypass with resultant diminished faecal losses (Phillips and Giller, 1973), but fat is not absorbed at all within the colon and faecal fat loss remains high (Althausen et al, 1950). The adaptive effects of different large and small bowel surgical manipulations on the human colon have received scant attention. There has been a single report of significant morphological atrophy demonstrated in the colon excised from a patient 30 years after it had been defunctioned by an ileostomy (Shaw-Dunn and Wright, 1981).

Other modulators of colonic adaptation

As in the small bowel, mucosal proliferation in the colon is sensitive to changes in food intake. **Bulk**, rather than nutrient content, appears to be the most important dietary variable governing the rate of epithelial cell turnover in the large bowel. A non-absorbable, non-nutritive **bulk diet** preserves colonic mucosal mass in the rat (Dowling et al, 1967). Conversely, **low residue** diets even if isocaloric with standard diets, produce colonic mucosal hypoplasia and a reduced rate of cell renewal (Janne et al, 1977; Morin et al, 1980; Heitman et al, 1983). By contrast, **starvation** reduces both total cellularity and the size of the proliferative compartment in the small bowel (Chapter 6) but in the colon these indices are unchanged (Hagemann and Stragand, 1977).

Other luminal constituents which might influence colonic cell proliferation include **mineral salts** (Stragand and Hagemann, 1978) and **bacteria**. In the rat colon, infection with *Citrobacter freundii* can induce non-specific hyperplasia (Barthold and Beck, 1980).

The control of colonic adaptation

Both luminal and systemic mechanisms may be involved in the control of adaptation in the large bowel as in the small bowel. As **nutrients** are usually completely digested and absorbed in the small bowel, they are unlikely to be directly tropic to colorectal mucosa (Bristol and Williamson, 1984). For the reasons outlined above, **bulk** seems to be the crucial luminal factor governing colorectal adaptation. **Endogenous secretions** such as bile and gastric and pancreatic juices could also be important in regulating colonic growth but there is, as yet, no convincing evidence for this. In the small bowel **enteroglucagon** seems the most likely gut peptide to be tropic

to the mucosa (Chapter 6), but its effect on the large bowel is uncertain. Raised enteroglucagon levels produced by small bowel resection had no effect on the epithelium of colonic loops isolated from the faecal stream (Bristol et al, 1982a). **Gastrin** appears to have no appreciable physiological role in the jejunum or colorectum (Bristol and Williamson, 1984). **Epidermal growth factor**, on the other hand, has been shown recently to have a tropic effect in colonic crypts (Al-Nafussi and Wright, 1982). The influence of possible antitropic peptides such as **somatostatin** on the colon is unknown.

Adaptation and carcinogenesis

Cell proliferation is an essential prerequisite for carcinogenesis (Cayama et al, 1978). It follows that a stimulus which increases the rate of cell division in a tissue might also increase its susceptibility to malignant change. Intestinal adaptation may be regarded as a normally favourable physiological mechanism which enables the organism to compensate for the loss of functioning tissue or dietary stress. Under certain circumstances however, increased cell proliferation might also predispose to cancer (Williamson, 1979) by increasing the number of intestinal epithelial cells exposed to a carcinogen (Richards, 1977). Alternatively it could "fix" any mutation by allowing rapid division of transformed cells and the establishment of a clone (Cayama et al, 1978).

Apart from an occasional case report, no direct association has yet been found in man between intestinal operations and subsequent colorectal carcinoma. However, there is a growing body of evidence accumulating from animal experiments supporting the hypothesis that intestinal adaptation predisposes to intestinal cancer. Over the last 5-6 years, various

operations found to stimulate adaptive hyperplasia in animals have been the subject of considerable investigation regarding possible effects on experimentally-induced intestinal carcinogenesis.

Varying degrees of **small bowel resection** (both proximal and distal) enhance the development of colorectal cancer induced by dimethylhydrazine or azoxymethane (Williamson et al, 1978d, 1980a,b; Oscarson et al, 1979; Hart et al, 1982; Williamson, 1982a). Subtotal enterectomy produces a modest increase in small bowel tumours compared to controls but no significant change in large bowel tumour yield possibly because of the profound weight loss after this operation (Williamson et al, 1980b). Besides enterectomy, and despite initial reports to the contrary, **enteric bypass** may also enhance carcinogenesis (Bell et al, 1982). Subtotal (85%) jejunoileal bypass enhances colorectal tumour yield despite loss of body weight of up to 40% (Bristol et al, 1984) and less extensive proximal jejunoileal bypass has recently been found to promote carcinogenesis to the same extent as the equivalent resection (Scudamore and Freeman, 1983). **Diversion of pancreaticobiliary secretions** to mid small bowel also enhances colorectal carcinogenesis (Williamson et al, 1979). Interestingly, tumours developed in the proximal small bowel segment in this model which, together with the observation that experimental tumours can develop in defunctioned colon (Wittig et al, 1971; Campbell et al, 1975) supports the view that azoxymethane and related compounds reach the bowel via the bloodstream.

Enteric resection and bypass and pancreaticobiliary diversion all increase the amount of bile and pancreatic juice entering the distal gut. Bile acids are cocarcinogenic when instilled intrarectally in rats (Narisawa et al, 1974; Reddy et al, 1976c, 1977b) and increased exposure of colorectal mucosa to them might explain the cocarcinogenic effect of these

operations. However, enterectomy has been found to increase the yield of tumours proximal to the line of resection, a region susceptible to adaptation but not exposed to increased levels of luminal constituents (Williamson, 1982a). Thus the effect of these procedures is more likely to be related to the adaptive response which they elicit.

Partial colectomy does not significantly enhance large bowel tumour yield except at suture lines, perhaps because colectomy has a limited role as a stimulus for adaptive growth of the remaining gut (Williamson et al, 1982). Subtotal colectomy, however, does produce a modest increase in rectal tumour yield (Williamson, 1982a). Permanent transverse **colostomy** partly protects the left colon from experimental cancer presumably due to mucosal atrophy (Campbell et al, 1975). By contrast, creation and subsequent closure of a transverse colostomy produces a marked growth spurt immediately after closure in the distal colorectal mucosa and actually enhances carcinogenesis even when the carcinogen is given immediately after colostomy closure when the left colon is still severely hypoplastic (Terpstra et al, 1981). Clearly the number of proliferating cells is more important in susceptibility to carcinogenesis than the total number of epithelial cells.

In summary, the experimental evidence to date supports the contention that experimental carcinogenesis is enhanced by the various surgical manoeuvres which produce adaptive hyperplasia. Conversely, in the colon at least, it may be depressed by stimuli which lead to mucosal hypoplasia.

CHAPTER 8

BILE ACIDS AND BACTERIA IN COLORECTAL CARCINOGENESIS

Bile acids

Bile acids are strong candidates for the role of endogenous cocarcinogens in colorectal cancer (Reddy, 1981; Thompson, 1982). They bear a close steric resemblance to an established group of carcinogens, the polycyclic aromatic hydrocarbons. Human gut flora can cause partial aromatization of the steroid ring and full conversion to 3-methylcholanthrene may be achieved by a series of simple chemical reactions (Hill, 1971). Deoxycholic acid receptors have been identified in human colonic cancers (Summerton et al, 1982).

Faecal excretion of bile acids is greater in populations from high-risk countries (Western Europe, USA) as opposed to low risk populations (Africa, Asia) (Aries et al, 1969; Hill et al, 1971). Moreover, bile acids are more likely to have undergone bacterial degeneration from the primary to the secondary form in these high risk groups (Thompson, 1982). Initial case-control studies suggested that patients with recognised cancer precursor conditions such as ulcerative colitis and adenomatous polyps, or with early cancer, excreted higher concentrations of bile acids than controls (Hill et al, 1975; Reddy and Wynder, 1977; Thompson, 1982). Furthermore, this increased concentration of faecal bile acids was linked to increased carriage of bacteria capable of degrading them to possible carcinogenic precursors (Hill et al, 1975). However, other investigations have failed to confirm these findings (Watne et al, 1976; Moskovitz et al, 1979; Mudd et al, 1980; Murray et al, 1980).

The level of faecal bile acid excretion depends on the amount of the bile acid pool lost during digestion and is related to the diet (Thompson, 1982). The effect of the dietary factors held to be most relevant in colorectal carcinogenesis (fat, protein and fibre), on faecal bile acid

excretion has been examined by dietary manipulation of healthy volunteers and in animals. High fat diets increase the total excretion of bile acids in man (Antonis and Bersohn, 1962; Hill, 1974) and the rat (Reddy et al, 1974a, 1977a). The proportion of secondary bile acids excreted is also increased (Nigro et al, 1976). Decreasing dietary fat intake in man for 1-4 weeks reduces faecal bile acid concentrations (Hill, 1970; Cummings et al, 1978). Although vegetarians have much lower faecal bile acid concentrations than meat eaters (Aries et al, 1969; Reddy and Wynder, 1973) short-term reduction of the protein intake of volunteers on a normal Western diet has a minimal effect on bile acid levels (Cummings et al, 1979). Increasing dietary fibre intake reduces bile acid concentration by producing bulkier stools but total bile acid loss is unchanged (Cummings et al, 1976; McLean Baird et al, 1977).

In experimental animals both acid and neutral sterols can induce sarcoma at injection sites (Reddy et al, 1980), though in this context they may be regarded as initiators rather than promoters of cancer. Oral administration of primary bile acids has promoted colorectal carcinogenesis in various models: rats and mice given dimethylhydrazine (Martin et al, 1981), or methylnitrosourea (Cohen et al, 1980), and rats with "spontaneous" cancers arising at a colostomy (Sauer et al, 1980). Binding agents such as cholestyramine and aluminium hydroxide that increase bile acid excretion may or may not enhance chemical carcinogenesis (Nigro et al, 1973; Asano et al, 1975; Cruse et al, 1981).

Primary and secondary bile acid solutions instilled per rectum directly into the colonic lumen also promote experimental carcinogenesis (Narisawa et al, 1974; Reddy et al, 1977b).

The mechanism of action of bile acids in colonic carcinogenesis has

not been elucidated. They might directly damage the epithelial cell; lithocholic acid can induce DNA strand breaks in cultured cells (Kulkarni et al, 1982). Alternatively they might stimulate hyperplasia thus rendering the mucosa more susceptible to neoplastic change (Chapter 7). Bile acids are thought to be tropic to ileal mucosa (Williamson et al, 1978c), and varying degrees of small bowel resection and pancreaticobiliary diversion to mid small bowel (PBD) produce moderate mucosal hyperplasia in the rat colon (Williamson et al, 1978c; Williamson, 1982b). By contrast, Miazza and coworkers (1982) suggest that bile acids are antitropic to the gut. They have found that PBD produces hyperplasia in the jejunum: the segment deprived of pancreaticobiliary secretions

Bacteria

A role for bacteria in carcinogenesis is suggested by the observation that cancer tends to arise in bowel segments which are most heavily colonised; the achlorhydric stomach and the colorectum (Thompson, 1983). In general bacteria may be responsible for the local production of carcinogens from inactive dietary constituents.

The composition of the colonic microbial flora is implicated as the key intermediary modulating the carcinogenic potential of a particular diet (Aries et al, 1969; Hill, 1979).

High-fat, low-protein diets increase the total anaerobic microflora in the faeces (Reddy et al, 1975a) as well as the activity of bacterial beta-glucuronidase (Reddy and Wynder, 1973; Reddy et al, 1975a; Goldin and Gorbach, 1976). The concentration of faecal anaerobes in one high-risk population (British) exceeded that of a low-risk population (Ugandan) especially for those bacteria capable of degrading bile acids (Aries et al,

1969; Hill et al, 1971). Nuclear-dehydrogenating clostridia in particular are capable of producing unsaturated steroids from the bile acid nucleus (Hill, 1974). These organisms may be more numerous in the faeces of patients with colorectal cancer than in control groups (Hill, 1975; Murray et al, 1980). Likewise bacterial enzymes that degrade primary bile acids to the secondary form may be more plentiful (Mastromarino et al, 1978). However, other studies have revealed no consistent differences in the number or type of bacteria between groups at varying risk (Moore and Holdeman, 1975; Finegold et al, 1975), so the metabolic activity of the microbial flora may be more relevant than the actual numbers of individual species (Reddy et al, 1980).

Since the cocarcinogenic activity of intrarectal bile acid solutions in germ free rats is diminished but not abolished, interaction with bacteria is clearly not essential (Reddy et al, 1976c,d, 1977b).

Substrates other than bile acids may be metabolised by bacteria to potential carcinogens. The close correlation of protein intake with colorectal cancer may be attributable to bacterial elaboration of carcinogenic metabolites from the amino acids tryptophan (Chung et al, 1975), tyrosine (Thompson, 1983) and methionine (Weisburger, 1971; Hill, 1979). Bacterial production of ammonia might also be important (Vissek et al, 1978).

Detoxification products of the widespread environmental carcinogens, the polycyclic aromatic hydrocarbons, may be converted to carcinogenic derivatives by gut clostridia and *E. coli* (Renwick and Drasar, 1976). Some gut bacteria may catalyse the formation of carcinogenic N-nitroso compounds from appropriate substrates in the large bowel (Thompson, 1983).

Viable cultures of *Lactobacillus acidophilus* fed to rats prevent the

promotional effect of a beef diet (Goldin and Gorbach, 1980), which may explain the low incidence of large bowel cancer in Finns who consume more milk and dairy products than their more susceptible Scandinavian neighbours (I.A.R.C., 1977). Moreover a high luminal pH is necessary for optimal degradation of bile acids so that acidification of the colonic lumen by fibre and milk could have a protective effect (Thornton, 1981).

The precise importance of bacteria in colorectal carcinogenesis is unclear. Germ-free rats remain susceptible to azoxymethane and dimethylhydrazine-induced carcinogenesis though tumour yields are usually reduced (Reddy et al, 1974b, 1975b, 1976c,d). Different types of bacteria may have widely differing and contrasting roles. Despite the alleged involvement of anaerobes in the conversion of bile acids to more active products, long-term oral administration of the potent anaerobicide, metronidazole to rats has been reported to have a promotional effect on chemical carcinogenesis (Sloan et al, 1983). By contrast, both tetracycline and erythromycin inhibit carcinogenesis in the rat (Goldin and Gorbach, 1981).

The role of bacteria in colorectal carcinogenesis may in part be related to an effect on intestinal cell kinetics. A normal physiological flora is necessary to maintain normal mucosal cell turnover in the colon as well as in the ileum where mucosal hypoplasia is seen in germ-free rats (Abrams et al, 1962; Williamson, 1978).

CHAPTER 9
MATERIALS AND METHODS

Materials and Methods

Animals

Young male Sprague-Dawley rats were employed in all experiments and were obtained from Olac SD, Bicester, Oxon, UK. This is a hardy outbred laboratory strain which stands up well to abdominal surgery and which is susceptible to azoxymethane-induced intestinal carcinogenesis.

Rats were received into the animal house at least one week before the start of each experiment. They were fed standard rat chow (Oxoid Breeding Diet; H.C.Styles & Co.Ltd., Bewdley, Worcs, UK) and water ad libitum and were housed 3-6 to a cage depending on size.

Surgical operations

All surgical operations were carried out under light ether anaesthesia. The various techniques employed are described in the next chapter. Laparotomy was always carried out through a midline incision which was closed with continuous Dexon. Anastomoses were fashioned in one layer using a continuous 6/0 silk suture. Animals were allowed free access to food and water immediately following surgery.

The small bowel was measured from the ligament of Treitz to the ileocaecal valve by gently stretching 5 cm segments against a ruler. In young rats the total length of the jejunoileum (in vivo) was ca. 90-100 cm. At sacrifice by 5-9 months of age, this segment measured ca. 110-150 cm after removal from the body. The duodenum was generally about 12 cm long at post mortem, and the entire colorectum varied from 20-35 cm in length.

At the end of each operation 0.25 mg Vitamin K was administered i.m. to prevent the troublesome postoperative bleeding previously reported in young Sprague-Dawley rats (Bristol et al, 1982b).

Carcinogen

Azoxymethane was the sole carcinogen used in this work and was supplied by Ash Stevens Inc., Detroit, Michigan, USA. On receipt from the manufacturer, the liquid carcinogen was diluted with sterile water. The resulting solution was stored at -20°C until required, when aliquots were thawed and further diluted with water. In experiments A and B, azoxymethane was administered by intraperitoneal injection of a volume of 1-2 ml containing a dose of 15 mg/kg body weight. Animals in experiments D and F received a similar dose injected subcutaneously into the flank. Injections are more easily administered intraperitoneally than subcutaneously by the inexperienced handler, but this advantage is offset by the greater risk of perforation of a viscus with subsequent death of the animal. Accordingly the subcutaneous route was employed in the later experiments. The incidence and distribution of primary and secondary tumours was unaffected by the route of administration. Injections were carried out at weekly intervals for 6 weeks resulting in a total dose of 90 mg/kg body weight.

Development of tumours

Animals were weighed weekly throughout the course of the experiment and daily for the first seven postoperative days. Some rats developed signs of intestinal cancer early in the experiment (3-4 months after carcinogen). Features such as progressive weight loss, haematochezia, anaemia and abdominal distension prompted early sacrifice on humane grounds. Most animals remained overtly healthy until the end of the experiment 20-30 weeks after the first injection of carcinogen.

Rats were killed by placing them in an ether chamber until apnoeic

followed by cervical dislocation. At autopsy, the thoracic and abdominal cavities and the auditory canals were carefully examined for evidence of primary or secondary neoplasia. The entire intestinal tract was excised, flushed clean with saline, opened along its antimesenteric border and examined by naked eye for tumours. Palpation usually resolved the occasional difficulty of distinguishing between a lymphoid follicle and a small tumour especially in the small bowel. The position of any obvious or suspected tumour (i.e. the measured distance from the distal end of the segment) was noted, together with its diameter or largest dimension. Tumours were then excised and fixed in 10% formalin for subsequent histological processing.

Distribution of intestinal tumours

By the time of sacrifice nearly all rats which received carcinogen had developed one or more intestinal tumours the majority of which (73%) were found in the colon (Figure 9.1). The distribution of colonic tumours found in each experiment are detailed in the next chapter but, generally, more developed in the transverse and descending (64%) colon than in the ascending segment. Rectal tumours were relatively less common than in man and the distal small bowel and caecum were rarely involved. Small-bowel tumours nearly always arose in the proximal jejunum and duodenum.

Large bowel tumours were mostly polypoid and hardly ever circumferential. They were usually multiple, scattered along the colorectum in clusters of 2-3 (Figure 9.1). Up to 17 tumours of varying sizes were found in the same animal. Small-bowel tumours were much less common than large bowel tumours and tended to arise singly, although up to 6 separate primaries were sometimes found. They were often sessile and

ulcerated, but occasionally large 1-2 cm diameter polypoid lesions were found.

Early deaths from cancer (10-20 weeks) usually resulted from intussusception of a duodenal or jejunal polyp and consequent obstruction. One case of biliary obstruction from a duodenal cancer was found (Figure 9:2). Colonic obstruction tended to occur later and was always due to intussusception of a pedunculated polyp and never to an annular stricture. This difference from human colonic cancer might be explained by the greater mobility of the rat colon, which remains within the peritoneal cavity on a mesentery throughout its length.

Metastases

Tumour spread was generally lymphatic, occasionally transcoelomic and rarely bloodborne. Initially mesenteric lymph nodes and subsequently para-aortic nodes were commonly affected. Metastasis throughout the peritoneal cavity resulted in carcinomatosis peritonei and malignant ascites (Figure 9:3), and transdiaphragmatic spread to mediastinal lymph nodes was an occasional finding. Pulmonary secondaries and nodules on the surface of the liver were also occasionally seen. Intrahepatic secondaries were detected in a small number of animals (Figure 9:4).

Extra-intestinal tumours

The only extra-intestinal site of neoplasia in these experiments was the external auditory canal (Figure 9:5). These tumours were squamous carcinomas of the ceruminous glands. Tumours could be bilateral, growing to 2-3 cm in diameter. They tended to invade deeply into the soft tissue of the face and interfered with feeding, resulting in marked loss of body



FIG. 9:1 Tumours induced by azoxymethane in rat distal colon.

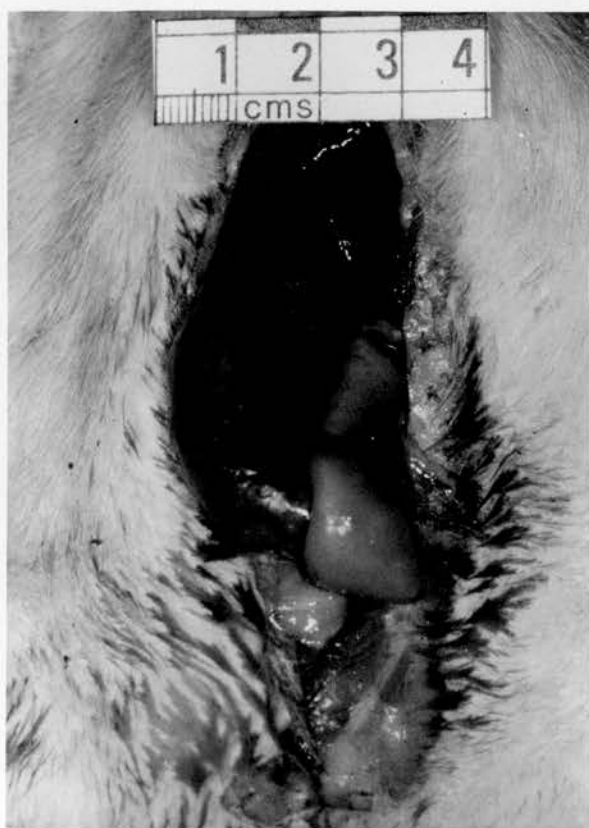


FIG. 9:2 Biliary obstruction by intersuscepting carcinoma of duodenum.

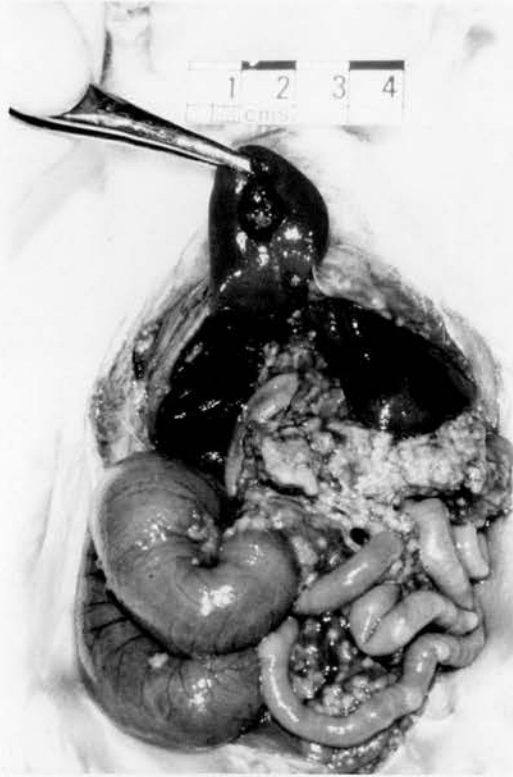


FIG. 9:3 Carcinomatosis peritonei

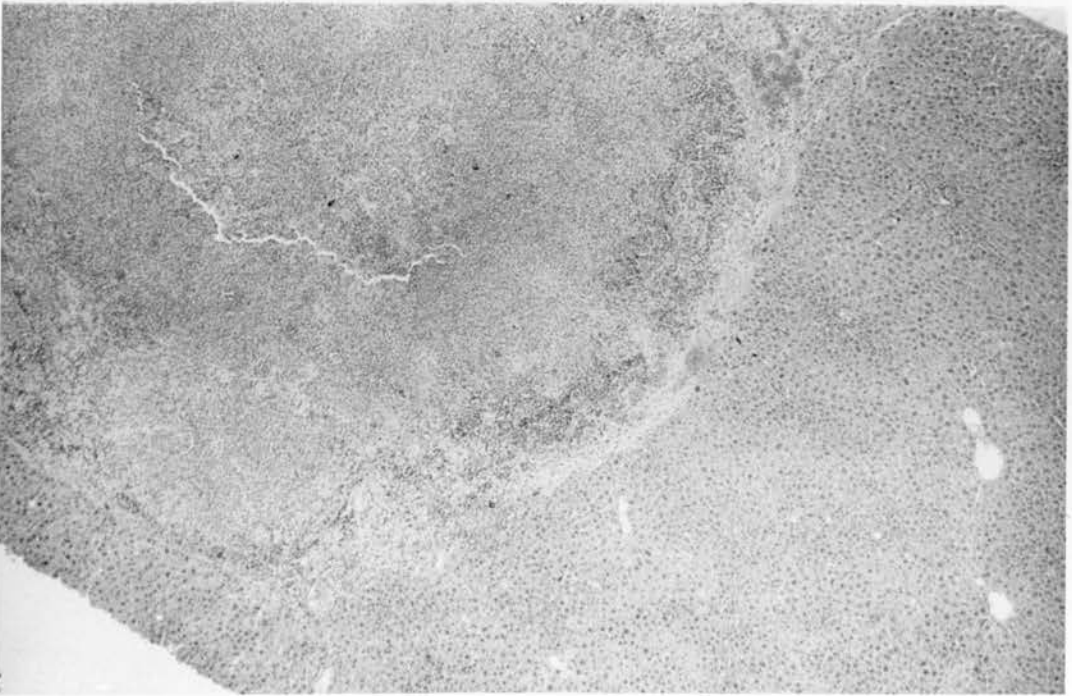


FIG. 9:4 Hepatic metastasis



FIG. 9:5 Tumour of external auditory canal.

weight. All animals with this lesion were killed humanely at this stage before death from starvation or bleeding supervened.

No tumours developed at the injection site or at any other extra-intestinal site in these experiments.

Pathology of tumours

Tumours were processed and imbedded in paraffin wax blocks from which 5µm thick sections were cut. Three to four sections from each tumour were stained with haematoxylin and eosin prior to examination under the light microscope.

Intestinal neoplasms were classified into three histological types. (a) Early lesions, mostly adenomas, but including foci of severe dysplasia, and hyperplastic polyps none of which revealed any evidence of invasion into the stalk or through the muscularis mucosae (Figure 9:6a,b). (b) "Intestinal" type adenocarcinomas showing papillary, tubular or mixed histological patterns and which invaded the base of the stalk (pedunculated lesions) or through the muscularis mucosae (sessile lesions) (Figure 9:6c-e). (c) Mucinous (colloid) adenocarcinomas characterised by "signet-ring" cells and which were all highly invasive (Figure 9:6f).

Macroscopic tumours varied in size from 1 to 35 mm in diameter, but size alone gave no clear indication of malignancy. Overall, invasive adenocarcinoma was the commonest histological type (51%). Much less common (10%), mucinous adenocarcinomas mostly arose in the duodenum, were all deeply invasive and tended to metastasize widely. Early lesions accounted for the remainder of tumours found (39%).

The proportions of the different histological types found were similar in all four carcinogen experiments described in the next chapter, and no differences were found between individual groups in each study.

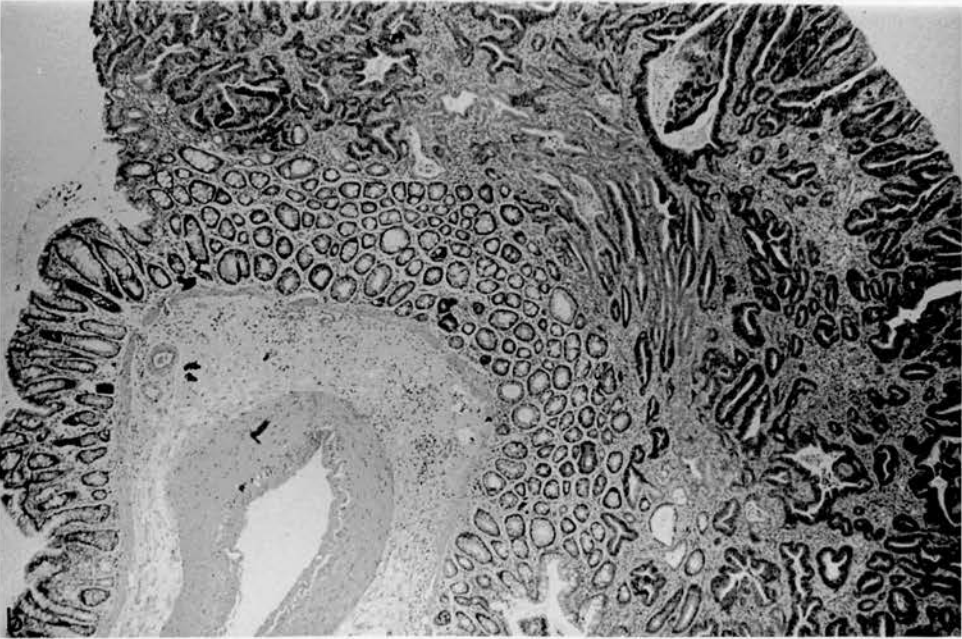
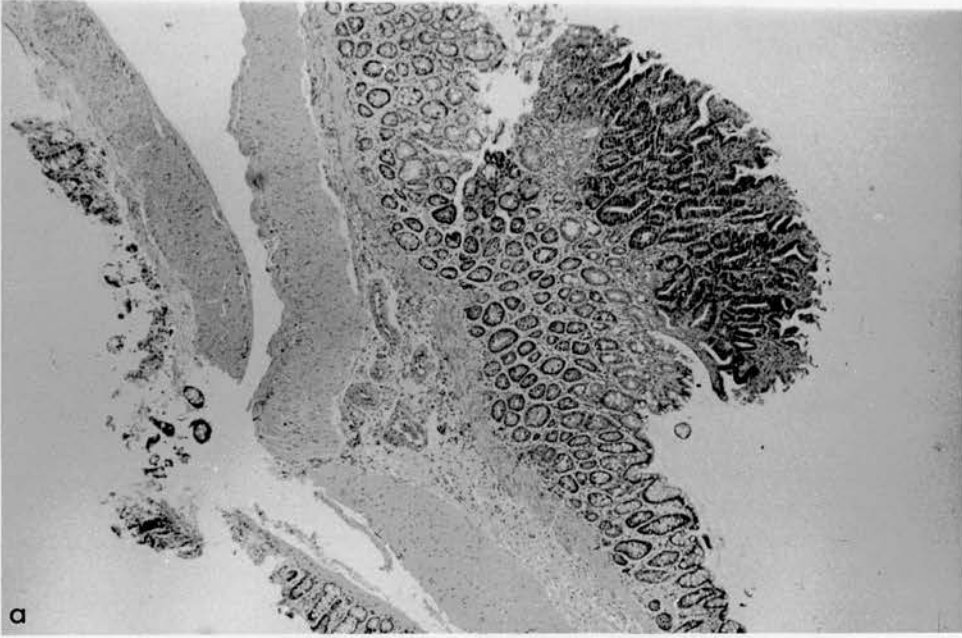


FIG. 9:6 a-b Non-invasive tumours with foci of dysplasia

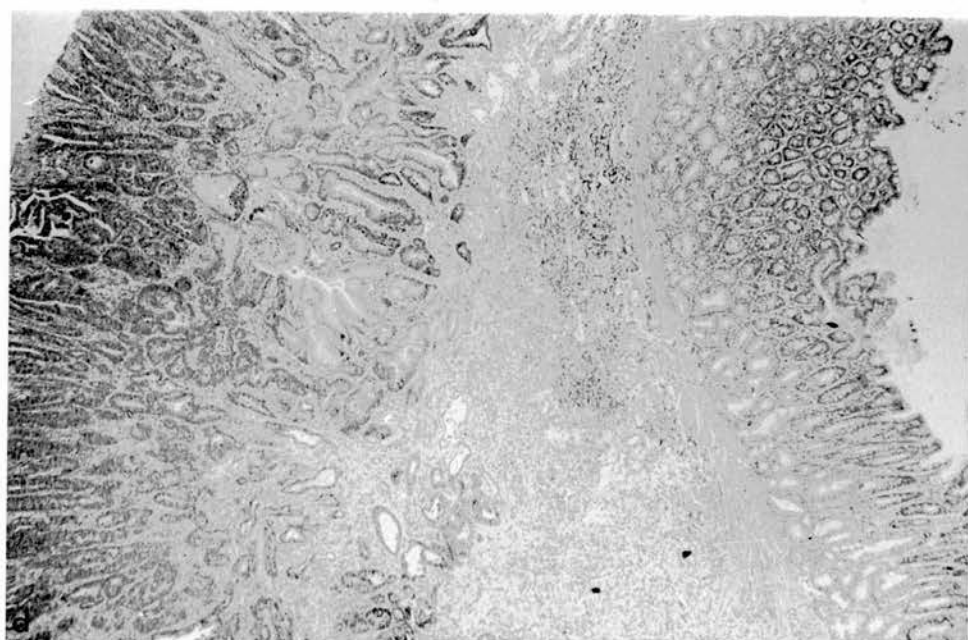
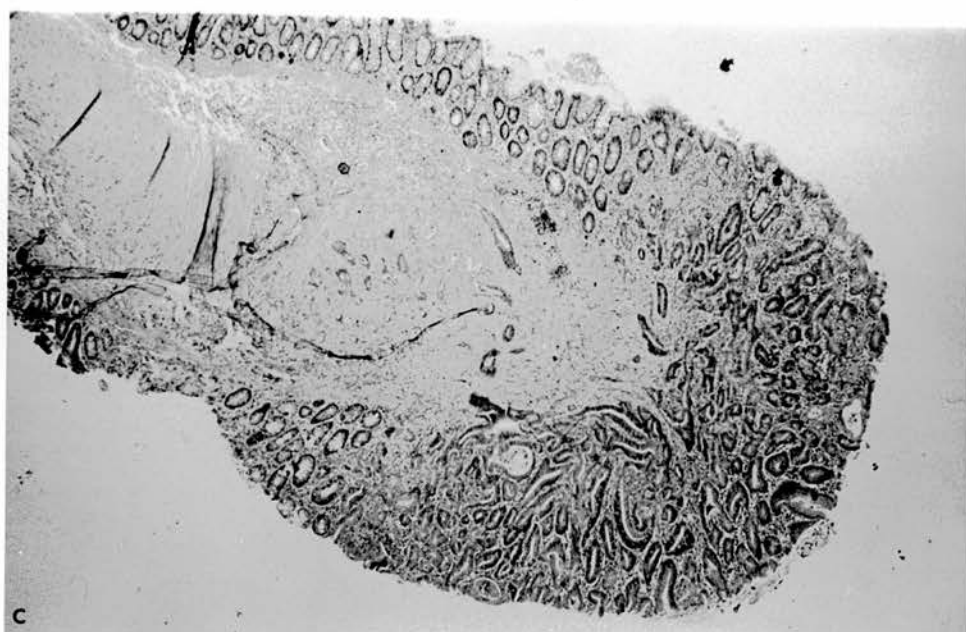


FIG. 9:6 c-d Invasive adenocarcinoma

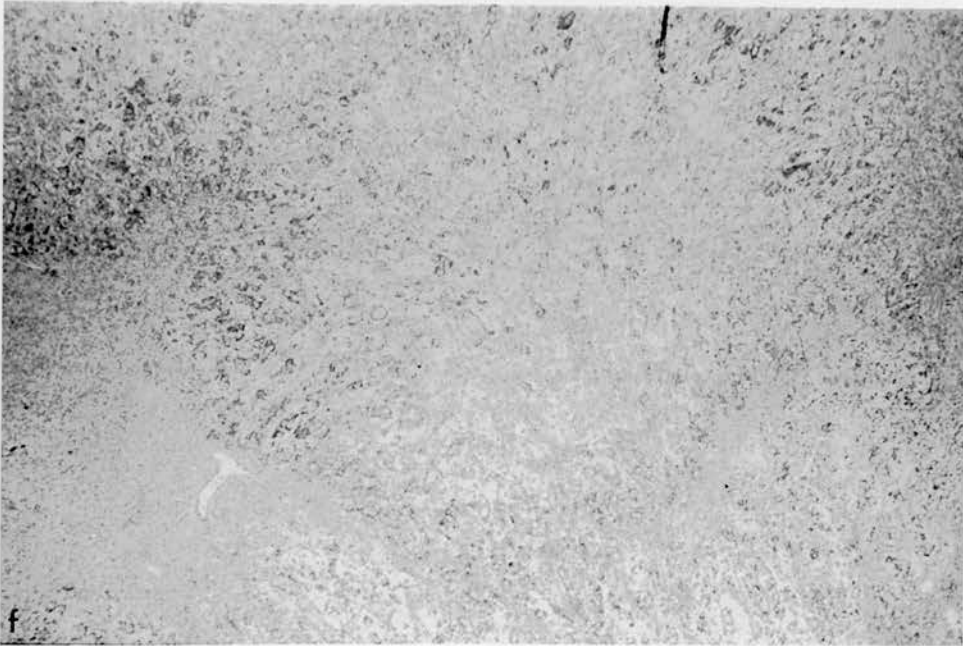
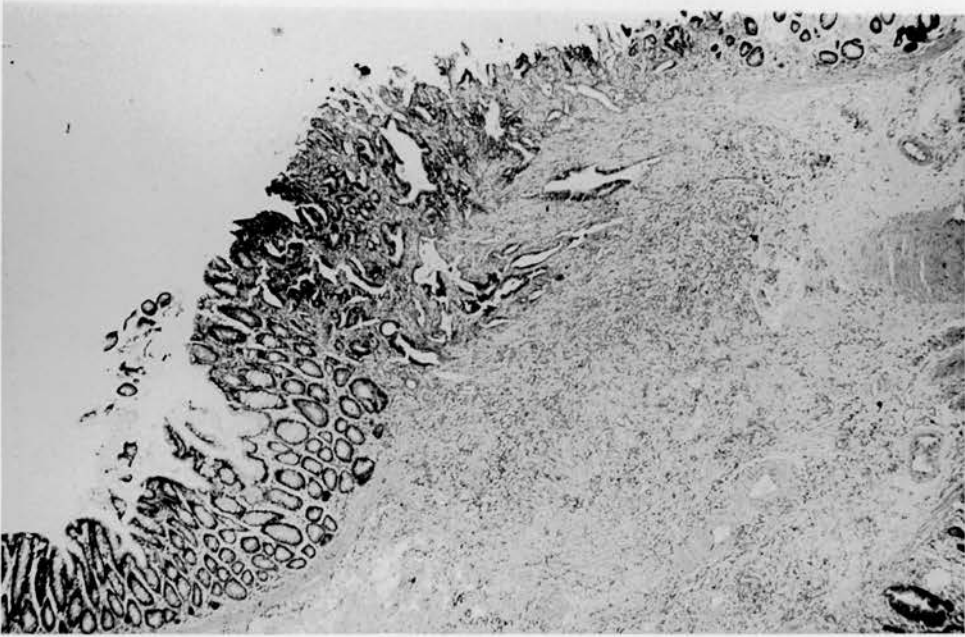


FIG. 9:6e Invasive adenocarcinoma
9:6f Mucinous adenocarcinoma

Intestinal adaptation

Length and Weight

Because intestinal adaptation affects all layers of the bowel wall, measurements of the length and wet weight of bowel segments are simple if crude indices of their proliferative state. These parameters were determined following excision at autopsy for the different bowel segments in each experiment. Segments were weighed after excision of all tumours. The length was measured by suspension with a constant weight (9.5g) against a ruler. The length of the caecum was not recorded owing to its variable shape. After weighing, it was spread out flat onto graph paper, and its outline was traced. As the weight/area ratio of the paper was known, the surface area of the caecum could be calculated from the weight of the cut-out outline.

Morphometry

Estimation of the mean mucosal crypt depth, which reflects gross alterations in mucosal cellularity (Williamson et al, 1978a), was carried out in experiments B, and D. In each case a 1 cm segment was excised from the relevant region of bowel and processed to 5 μ m sections stained with haematoxylin and eosin. Under light microscopy, using an ocular micrometer, 10 properly orientated crypts were measured per section.

Crypt Cell Production Rate

The simple morphometric measurement of crypt depth has basic theoretical limitations as an index of the mucosal proliferative state (Al-Mukhtar et al, 1982). In the intestine, the fundamental parameter of mucosal proliferation is the birth rate of new cells in the crypt. In each

crypt, the cell production rate is influenced by three factors: the cell-cycle time, the growth fraction and the cell population size. Fluctuations may occur in these factors which result in altered cell production without corresponding morphometric changes. Theoretically, for example, the proportion of a cell population given over to proliferation (the growth fraction) might be increased, while cell cycle time and the cell population remain constant, resulting in a net increased cell production rate not reflected by any corresponding increase in crypt depth. Conversely reduced crypt depth as a result of a decreased cell population would not necessarily indicate decreased cell proliferation if the growth fraction was increased or the cell-cycle time shortened.

At present the most direct method of measuring the crypt cell production rate that is independent of these three governing factors is the stathmokinetic or metaphase arrest technique. This method was employed in four of the experiments detailed in the next chapter. In principle, this method involves the administration to a proliferating cell population of an agent which blocks cells in mitosis. The accumulations of arrested metaphases over a period of time enables the rate of entry of cells into mitosis to be calculated (the birth rate) (Wright and Appleton, 1980). In the case of gut epithelium this measurement is termed the crypt cell production rate (CCPR) and expressed as cells/crypt/hour.

In practice, six rats from each group to be studied were employed. They each received an intraperitoneal injection of the metaphase-arresting agent vincristine (1 mg/kg) at 0830 hrs on the day of sacrifice. Studies were always carried out between 0830 and 1130 hrs to avoid diurnal variations in intestinal cell kinetics (Williamson, 1978). The six rats were then killed at half-hourly intervals from 30-180 minutes after vincristine. Immediate

autopsy was performed, and the bowel was excised and cleaned as described above. Any tumours found were excised and fixed in formalin after their size and position had been determined. The small bowel was divided into three segments, proximal, middle and distal thirds, which were laid flat on strips of thin cardboard which were then rolled up and placed in clearly labelled jars containing the fixative (Carnoy's reagent). The cardboard backing was used to prevent excessive shrinkage and distortion of the tissue during fixation and storage. The caecum was spread out on cardboard and treated in the same fashion. The characteristic "herringbone" appearance of proximal colonic mucosa enabled easy identification and orientation of the different large bowel segments, and therefore the entire colorectum could be fixed as a single specimen. Specimens were left in the fixative for 4-6 hours before transfer to absolute alcohol in which they could be stored indefinitely.

Later (one week to several months) the rolled-up specimens were removed from the alcohol and opened out. Small 3-4 mm square samples were cut from the region to be examined. The original specimens were then replaced in the alcohol; subsequent repeat samples could be taken from them if necessary. In order to determine the crypt cell production rate in a particular segment, a sample of that segment was taken from each of the six rats in the group.

These small samples were then stained with Schiff's reagent by Feulgen's method. Each freshly stained sample was removed from the stain, rinsed with 45% acetic acid, placed on a microscope slide and observed under a dissecting microscope. Fresh disposable hypodermic needles (18 G) or sharpened dental probes were used to strip the mucosa from the underlying muscularis mucosae and muscle coat. It was then possible to

tease out individual crypts or paired crypts from the rest of the sample. In the case of the small bowel it was necessary to shave off the villi before attempting to dissect out the crypts. The main bulk of the sample was removed from the slide, leaving behind a small number of single crypts or pairs of crypts over which a coverslip was placed. The slide was then transferred to the stage of a light microscope; gentle pressure was exerted on the coverslip until the crypts were squashed flat enough to be clearly in focus in only one plane.

Feulgen's method is a specific histochemical reaction for DNA. Schiff's reagent reacts with aldehyde groups released from the DNA when the tissue is hydrolysed to produce a magenta colour. Arrested metaphases can be seen clearly in the microdissected crypts; they contrast with the pale pink of the non-dividing cells and can be counted (Figure 9:7).

The mean number of arrested metaphases per crypt for ten microdissected crypts per sample was determined and plotted against time of death after vincristine. The slope of the line fitted to this data by linear regression and least squares approximation represents the crypt cell production rate (cells/crypt/hour) for that segment of bowel (Figure 9:8). Using this technique the CCPR was generally determined for the following segments: proximal, middle and distal thirds of the jejunoileum, caecum, proximal middle and distal thirds of the colorectum.

In theory the stathmokinetic (metaphase arrest) method is simple, but, in practice, considerable skill and impeccable technique is required if good results are to be obtained. The important practical points in this method detailed by Wright and Appleton (1980) were followed throughout these studies. Nevertheless it is probably significant that differences observed between CCPR, in the later experiments (D,F) tended to be of greater

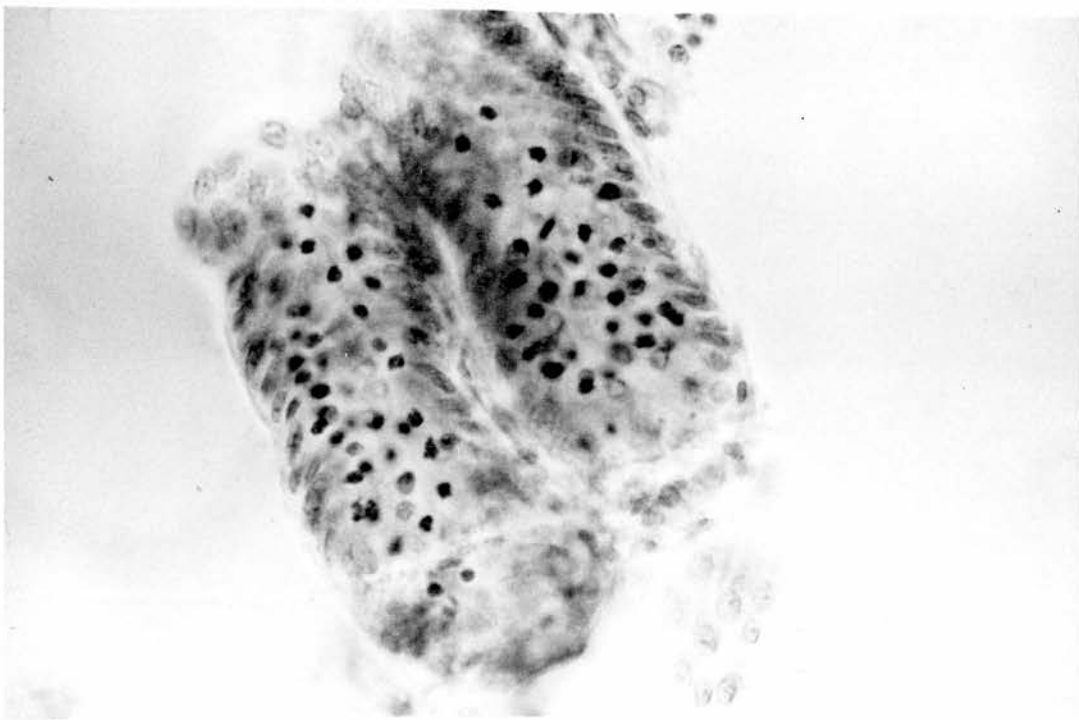


FIG. 9:7 Arrested metaphases in isolated colonic crypts

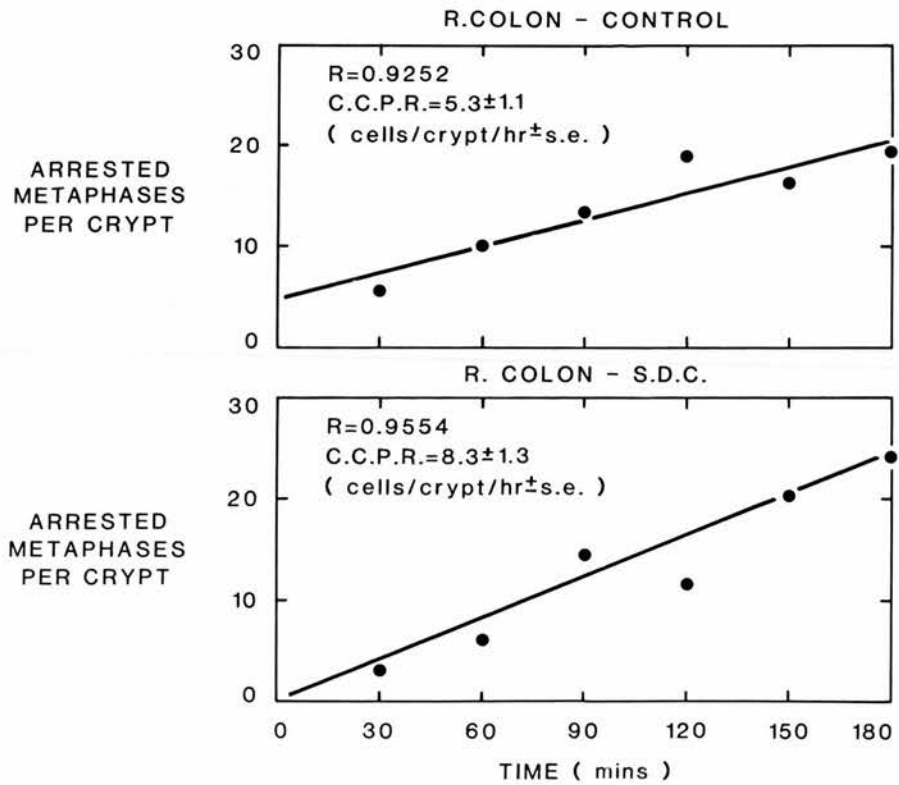


FIG. 9:8 Number of arrested metaphases per crypt plotted against time after sacrifice in two experimental groups (chapter 10:e). Crypt cell production rates calculated from slopes of least-squares regression lines.

statistical significance than those found earlier (Experiments A,C). Clearly, experience of the technique is a crucial factor in the quality of the data obtained. The best way to improve the data would probably be to use more animals and shorter time intervals, but in this work, these measures were precluded by financial and logistic constraints.

Statistics

Student's t-test was employed for evaluation of the body weight, tumour yield and morphometric data detailed in the next chapter.

The stathmokinetic technique produced interesting results but, despite its theoretical advantages over other cruder methods, many apparently convincing differences in CCPR between groups failed to prove statistically significant. On the advice of a professional statistician, pairs of CCPR regression lines were compared initially by pooling both sets of data and fitting a single regression line. The significance of the difference between their individual slopes was assessed by calculating the f -statistic, which indicated the degree to which two separate regression lines explained the data more fully than the single combined regression line. However, this test is not used in the Department of Histopathology at the Hammersmith Hospital where the stathmokinetic was developed to its present state and where the simpler, but more exacting, Student's t-test for comparison of regression lines is preferred (Wright and Appleton, 1980; Al-Mukhtar et al, 1982). Accordingly the CCPR data presented in this thesis has been analysed by this method.

Other techniques

The bacteriological methods employed in experiment E are described in that section.

CHAPTER 10
EXPERIMENTAL RESULTS

EXPERIMENT A

Intestinal adaptation and carcinogenesis after distal ileal resection and bypass.

Background

In man distal small bowel resection is often carried out in the management of Crohn's disease and distal ileal bypass has been advocated for the control of hyperlipidaemias (Buchwald et al, 1974). Surgical shortening of the gut stimulates compensatory hyperplasia of the remaining bowel, which allows the organism to adapt to the loss of functioning tissue (Williamson, 1982a,b). Under certain circumstances, however, this physiological response may also predispose to carcinogenesis. In the rat, partial enterectomy produces sustained mucosal hyperplasia in the remaining small bowel distal to the line of resection and to a lesser extent in proximal small bowel and colon (Nundy et al, 1977; Oscarson et al, 1979; Williamson et al, 1978a,d, 1980a). It also promotes chemically-induced intestinal carcinogenesis (Williamson, 1982a). Likewise, partial enteric bypass of sufficient extent will enhance carcinogenesis. Subtotal jejunoileal bypass results in structural adaptation of the colon and increased tumour yields, despite the loss of up to 40% of body weight (Bristol et al, 1982a, 1984). Less extensive small-bowel bypass operations have a slower but ultimately similar adaptive effect to resection; their effect on carcinogenesis is uncertain (Williamson et al, 1980b; Bell et al, 1982).

The present study was designed to compare the effects of resection and bypass of the distal small bowel on cell proliferation and carcinogenesis. How compensatory hyperplasia predisposes to carcinogenesis is not clear. The population of epithelial cells at risk of malignant transformation might

simply be increased, or alternatively cells which have undergone malignant change may be stimulated to divide more rapidly, thus reducing the delay between exposure to the carcinogen and the development of tumours (Williamson et al, 1980a). In an attempt to answer this question, one-third of the animals in each operative group were sacrificed at 20, 25 and 30 weeks postoperatively.

Design

One hundred and forty-five rats weighing 100-125 g were allocated to one of three groups (Figure 10a:1). Operations were performed at the start of the experiment (week 1). In group 1 (n=45), transection and reanastomosis of the small bowel at the junction of its middle and distal thirds was combined with caecotomy and resuture as a control operation (Figure 10a:2a). In group 2 (n=45), the distal 33% of the small bowel was bypassed (DSBB), and intestinal continuity was restored by anastomosing the proximal two-thirds of the small bowel end-to-side to the caecum (Figure 10a:2b). This operation is analogous to distal ileal bypass for hyperlipidaemia in man (Buchwald et al, 1974). Rats in group 3 (n=45) underwent resection of the distal 33% of the small bowel (DSBR) with end-to-side ileoneocaecostomy (Figure 10a:2c).

One week later rats received the first of six weekly i.p. injections of azoxymethane. Rats in each operative group were further randomised to three subgroups, A, B and C. Groups 1A, 2A and 3A were to be sacrificed at 20 weeks postoperatively, groups B at 25 weeks and groups C at 30 weeks (Figure 10a:1).

Rats were regularly examined for evidence of tumour development and were killed at 20, 25 or 30 weeks, or earlier if moribund. At autopsy the

entire intestinal tract was excised. The following segments were thoroughly flushed with cold saline to remove all content: duodenum, jejunum, caecum and colorectum. The length and wet weight of each segment were recorded. The weights of the liver, kidneys and spleen were also noted. Tumours were identified, excised and treated as described in Chapter 9.

In addition, at 20 weeks and 30 weeks 6 rats from each subgroup received the stathmokinetic agent vincristine 1 mg/kg i.p. Animals were then killed at half-hourly intervals from 30 minutes to 180 minutes after vincristine. At autopsy, the following intestinal segments were fixed in Carnoy's solution: proximal, middle and distal thirds of jejunum, caecum, proximal middle and distal thirds of colorectum. The distal jejunal specimen was from the bypassed loop after DSBB and was missing after DSBR. These segments were subsequently processed as previously described (Chapter 9) for estimation of crypt cell production rates (CCPR).

Results

Mortality

Thirteen rats (9%) died before the end of the first postoperative week, from haemorrhage or anaesthetic overdose. Another 22 animals died during the early part of the experiment from either mechanical obstruction at the anastomosis (12 rats), anastomotic leakage (6) or internal hernia (4). Between 6-8 weeks postoperatively, 4 rats (2 DSBB and 2 DSBR) died from gastrointestinal haemorrhage, possibly because of vitamin K malabsorption resulting from loss of functioning ileum (Williamson et al, 1980a). Further bleeding was prevented by a second i.m. injection of vitamin K 0.25 mg administered to all animals during week 8. Four rats in the DSBR group died prematurely from cancer: 2 had tumours of the external auditory canal

alone, and 2 had intestinal tumours at 14 and 16 weeks.

All rats dying before week 20 were excluded from the analysis. The 9 rats that died spontaneously thereafter were included in the overall analysis of tumour yields but not in the results obtained from the individual planned sacrifices at 20, 25 and 30 weeks. Overall, 91 animals (67%) survived until sacrifice (Figure 10a:1), the mortality rate being slightly higher in the DSBR group (40%) than in the DSBB (27%) and control groups (31%).

Body weight

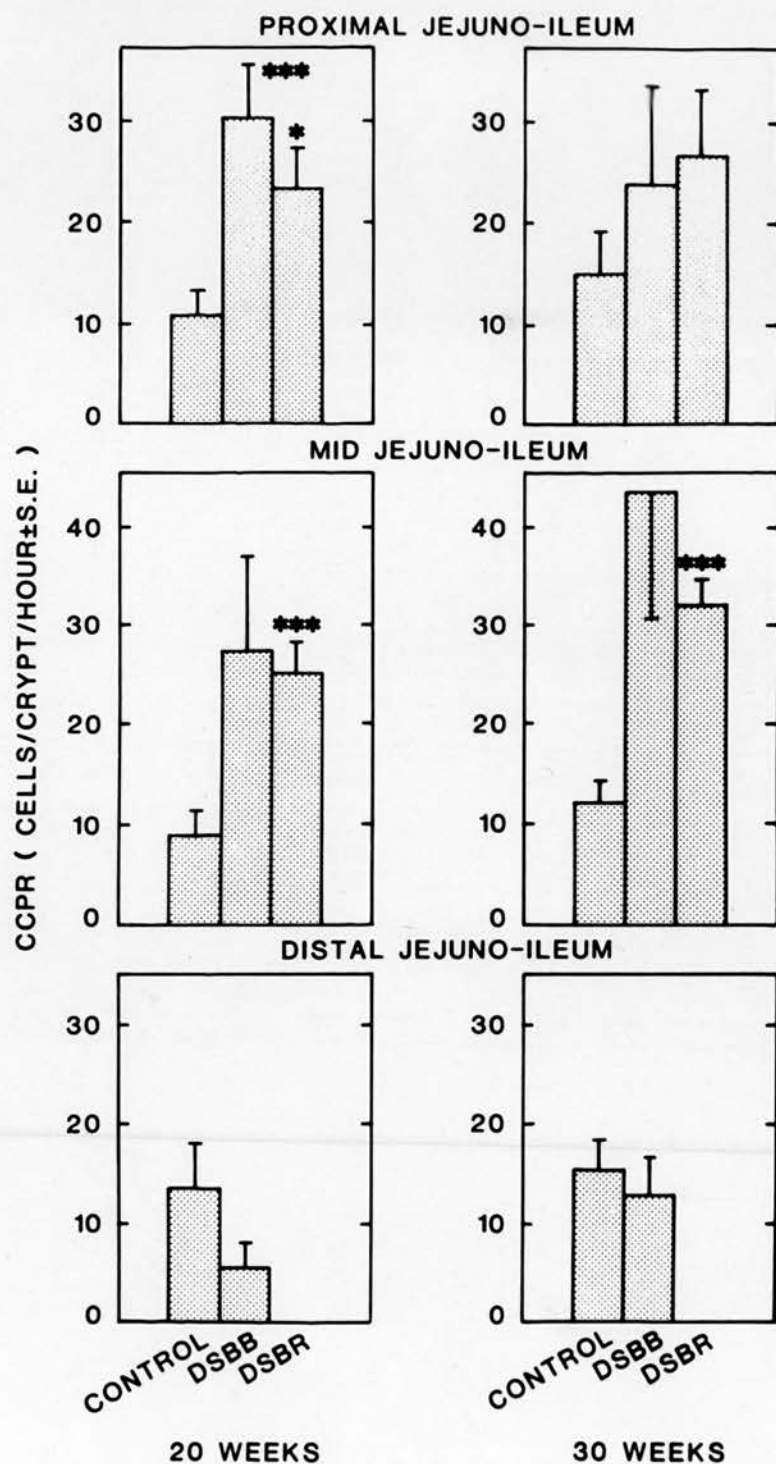
Within a week of operation mean body weights exceeded preoperative values to a similar degree in all three groups. From the 10th week onwards rats with DSBR gained weight more slowly than the others, and at the end of the experiment their mean weight ($506 \pm 15\text{g}$; \pm SEM) was 10% lower than the DSBB group ($560 \pm 12\text{g}$) and controls (568 ± 9) ($P < 0.01$).

Intestinal adaptation

No differences were found in the length and wet weight of the duodenum, colorectum or proximal two-thirds of the jejunioileum. After DSBB the excluded loop weighed only 66% of the equivalent segment in controls. The wet weight of the caecum was 53-65% higher after DSBR ($2.8 \pm 0.1\text{g}$) and DSBB ($2.6 \pm 0.1\text{g}$) than in controls ($1.7 \pm 0.1\text{g}$; $P < 0.05$).

At 20 weeks crypt cell production rates (CCPRs) in control large and small intestine varied between 8.2-13.5 cells/crypt/hour; rates after DSBB and DSBR were 115-109% higher in the functioning small intestine (Figure 10a:3) and up to 96% higher in the large intestine (Figure 10a:4) (Table 10a:1). By 30 weeks the gap had generally widened though not in the distal colon. Percentage increments were highest in the mid small bowel (159-

FIG.10a:3



20 WEEKS

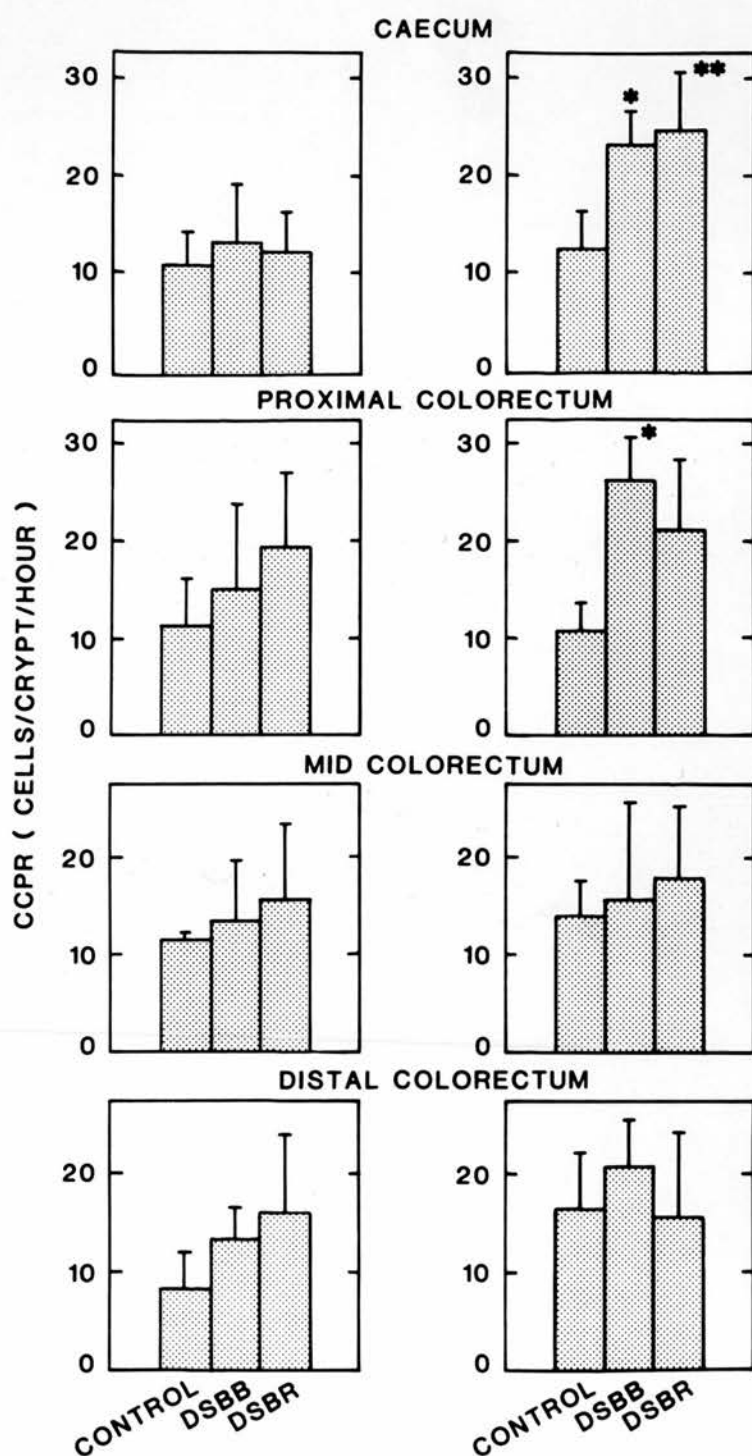
30 WEEKS

CRYPT CELL PRODUCTION RATES
(CCPR=CELLS/CRYPT/HOUR±S.E.)
IN SMALL BOWEL AT 20 AND 30 WEEKS

DSBB=DISTAL SMALL BOWEL BYPASS
DSBR=DISTAL SMALL BOWEL RESECTION

* $P < 0.05$ vs CONTROLS *** $P < 0.01$ vs CONTROLS

FIG.10a:4



20 WEEKS 30 WEEKS

CRYPT CELL PRODUCTION RATES
(CELLS/CRYPT/HOUR \pm S.E.)
IN LARGE BOWEL AT 20 AND 30 WEEKS

DSBB=DISTAL SMALL BOWEL BYPASS
DSBR=DISTAL SMALL BOWEL RESECTION
* $P < 0.05$ ** $P < 0.02$ vs CONTROLS

		<u>Controls</u>	<u>DSBB</u>	<u>DSBR</u>
Proximal Jejunioileum	20 weeks	10.8 \pm 2.4	30.4 \pm 5.4***	23.2 \pm 4.1*
	30 weeks	15.0 \pm 4.1	23.7 \pm 9.8	26.7 \pm 6.4
Middle Jejunioileum	20 weeks	8.9 \pm 2.5	27.5 \pm 9.5	25.1 \pm 3.0***
	30 weeks	12.4 \pm 2.1	43.4 \pm 12.8	32.1 \pm 2.8***
Distal Jejunioileum	20 weeks	13.5 \pm 4.5	5.5 \pm 2.7	
	30 weeks	15.4 \pm 2.9	12.9 \pm 3.9	
Caecum	20 weeks	10.9 \pm 3.3	13.1 \pm 5.9	12.1 \pm 4.1
	30 weeks	12.4 \pm 3.8	23.0 \pm 3.8*	27.3 \pm 3.2**
Proximal Colorectum	20 weeks	11.4 \pm 4.8	15.1 \pm 8.8	19.5 \pm 7.7
	30 weeks	10.9 \pm 2.9	26.3 \pm 4.5*	21.2 \pm 7.4
Middle Colorectum	20 weeks	11.5 \pm 0.7	13.5 \pm 6.1	15.7 \pm 7.6
	30 weeks	13.9 \pm 3.9	15.6 \pm 10.3	18.0 \pm 7.3
Distal Colorectum	20 weeks	8.2 \pm 3.7	13.3 \pm 3.2	16.1 \pm 8.1
	30 weeks	16.2 \pm 5.9	20.4 \pm 4.8	15.2 \pm 9.0

Table 10a:1 Crypt Cell Production Rates (cells/crypt/hour \pm S.E.) in controls and after Distal Small Bowel bypass (DSBB), and Distal Small Bowel resection (DSBR) at 20 and 30 weeks.

* $\underline{P} < 0.05$, ** $\underline{P} < 0.02$, *** $\underline{P} < 0.01$, versus controls.

250%) and proximal colon (32-141). Surprisingly, in the mid small bowel, increases of 159 and 182% after DSBR were statistically significant ($P < 0.01$), yet increases of 209 and 250% after DSBB were not (Figure 10a:3). Similarly, in the proximal large bowel only the 141% increase after DSBB at 30 weeks was significant ($P < 0.05$) (Figure 10a:4). CCPR in the caecum was initially slow to respond to DSBB and DSBR but at 30 weeks matched that of the proximal colon with increments of 85 ($P < 0.05$) and 120% ($P < 0.02$) over controls.

There was no consistent difference in the degree of adaptive hyperplasia to DSBR and DSBB at either 20 weeks or 30 weeks. The lowest CCPR (5.5 cells/crypt/hour) was observed in the bypassed loop at 20 weeks and was less than half that in the equivalent control segment, though this difference did not quite reach statistical significance.

Regardless of group, CCPRs tended to increase between 20 and 30 weeks except in the proximal colorectum of controls, the proximal small bowel after DSBB and the distal large bowel after DSBR (Table 10a:1).

Intestinal tumours

All but 1 or 2 rats in each group developed at least one colorectal tumour. Tumour yields tended to increase with time after exposure to carcinogen in control rats and those with DSBB, though after DSBR yields were already near maximal at 20 weeks (Figure 10a:5). The overall yield in the DSBR group (5.3 ± 0.5 tumours per rat; mean \pm SEM) was 55% higher than in controls (3.4 ± 0.5 ; $P < 0.02$). The overall yield in the DSBB group (4.5 ± 0.5) was 32% higher than in controls, but this difference did not quite attain statistical significance ($0.5 < P < 0.1$).

At 20 weeks the DSBR tumour yield was twice that of controls ($P <$

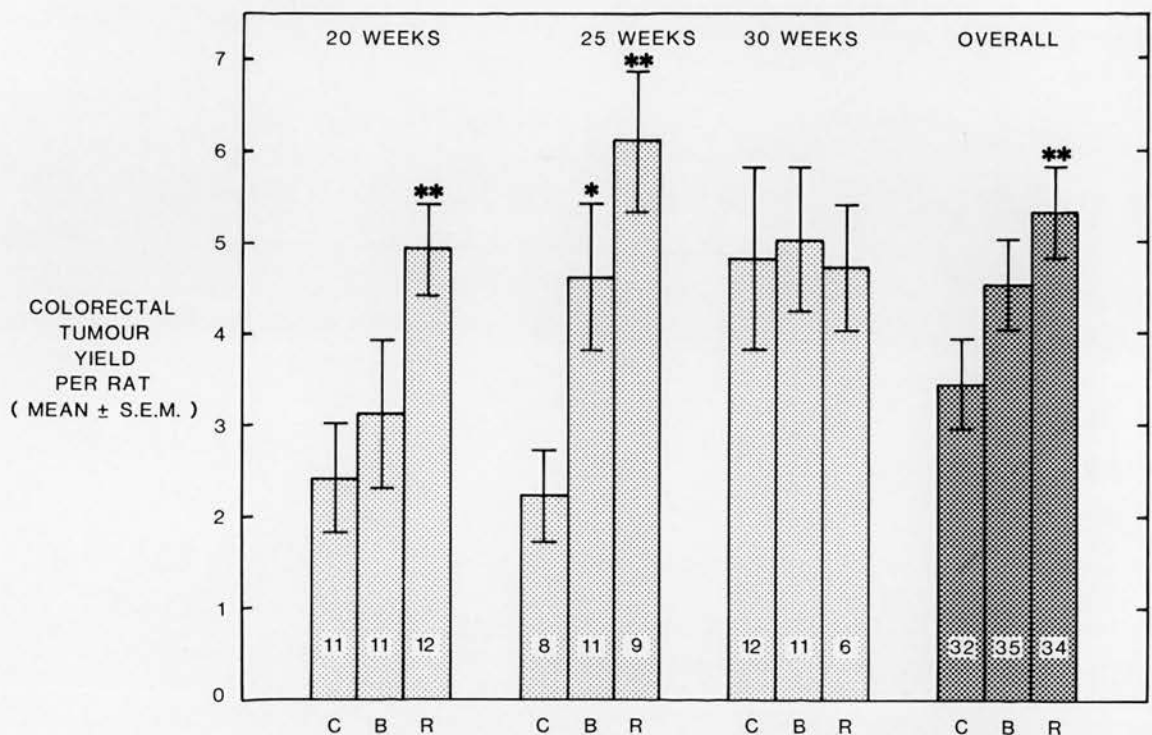


FIG. 10a:5 Colorectal tumour yields (mean \pm s.e.) both at staged sacrifices and overall. C=controls, B=distal small bowel bypass, R=distal small bowel resection. Numbers in each group are shown. Significance vs controls ** $p < 0.02$; * $p < 0.05$.

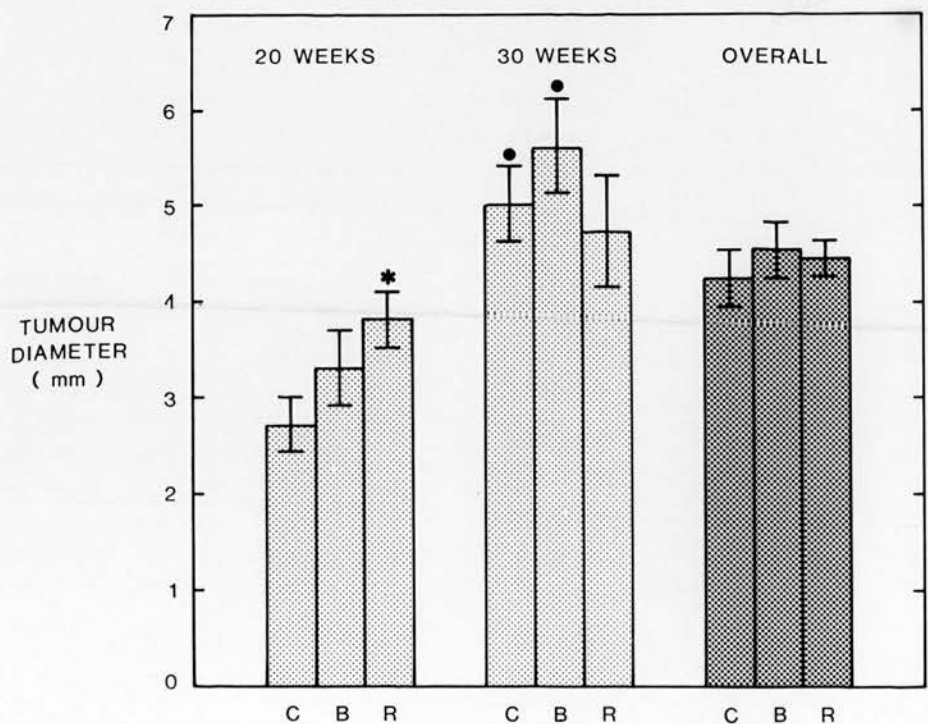


FIG. 10a:6 Colorectal tumour diameter (mm) (mean \pm s.e.) at 20 weeks and 30 weeks and overall. Significance: * $p < 0.02$ vs controls ° $p < 0.05$ vs 20 week values.

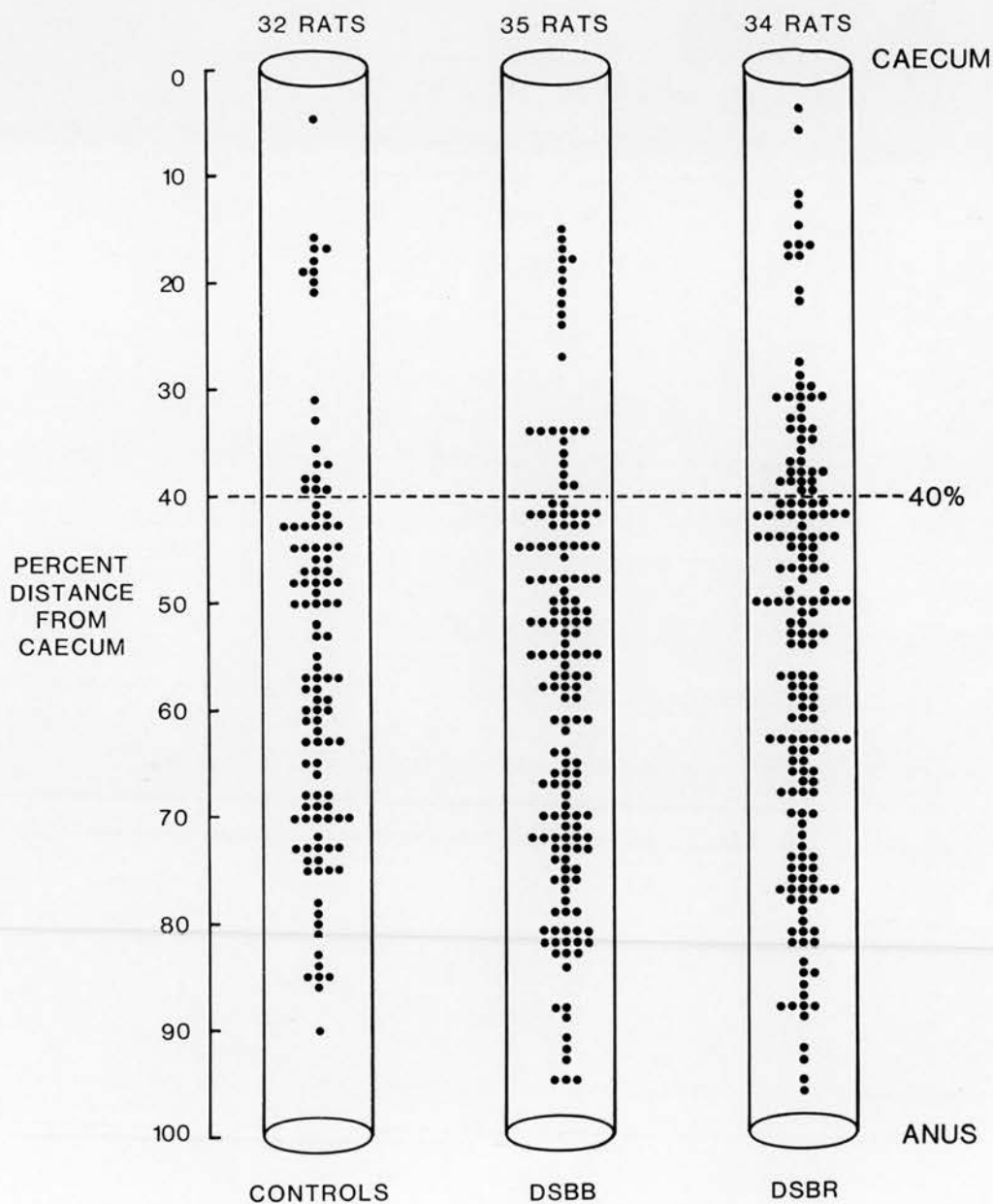


FIG. 10a:7 Colorectal tumour distribution after distal small bowel bypass (DSBB) and resection (DSBR).

.02) and 58% greater than after DSBB (Figure 10a:5). By 25 weeks the difference between DSBR and DSBB yields had narrowed to 33%; both operations produced more than twice as many tumours as controls. At 30 weeks, however, tumour yields were almost identical in the three groups.

At 20 weeks the mean tumour diameter in rats with DSBR (3.8 ± 0.3 mm) was 41% greater than that of controls and 15% greater than that of rats with DSBB (Figure 10a:6). These differences had disappeared by 30 weeks. Compared to the 20 week measurements mean tumour size had increased by 85% in controls and by 70% in the DSBB group ($P < 0.01$), but there was a much smaller increase (24%) in the DSBR group. Overall there were no significant differences in tumour size between the three groups.

The pattern of colorectal tumour distribution was similar in the three groups (Figure 10a:7): overall, 84% of tumours developed in the distal 60% of the large bowel. At 20 weeks the mean number of tumours found in the proximal 40% was 1.2 ± 0.1 per rat in the DSBR group, 0.5 ± 0.2 in the DSBB group ($P < 0.01$) and 0.1 ± 0.1 in controls ($P < 0.02$). The yield of proximal tumours was similar in all groups at 30 weeks, however.

Tumours arose in the duodenum ($n=63$) and jejunum (15), but their incidence was not affected by operation or time of death. In addition 4 rats had caecal tumours, and 12 had tumours of the external auditory canal. Irrespective of operation, metastases were commoner after 25 weeks; usual sites included lymph nodes and omentum, liver, lungs and pleura. Carcinomatosis peritonei developed in 4 rats.

Comment

This study confirms that distal enterectomy promotes experimental colorectal carcinogenesis and indicates that distal enteric bypass has a

similar but less pronounced effect. By contrast Scudamore and Freeman (1983) have recently found that proximal jejunoileal resection and bypass promote carcinogenesis to the same extent. The lesser effect of distal ileal bypass in promoting colorectal carcinogenesis cannot be explained by lower body weight. Although rats generally weigh less after bypass than after equivalent resection or transection (Williamson et al, 1980b), no weight differentials were observed in the present experiment, perhaps because preservation of the ileocaecal valve prevented gross bacterial colonisation of the blind loop. The lower body weight of rats with DSBR during the later stages of the experiment probably reflected their earlier development of intestinal cancers.

The crypt cell production rate data were generally disappointing. Several apparently clear differences between groups failed to show statistical significance probably due to large standard errors. Nevertheless both excision and exclusion of the ileum caused a substantial increase in intestinal crypt cell proliferation on either side of the line of resection. This compensatory hyperplasia occurred throughout the intestinal tract from the proximal jejunum to the distal colon, but changes were most marked and statistically significant in the mid small bowel and proximal large bowel. This pattern of a generalised adaptive response, which is maximal adjacent to the 'missing' segment, has been observed previously (Williamson et al, 1978a,d; Oscarson et al, 1979; Williamson, 1982b). After both resection and bypass intestinal growth continued between 20 and 30 weeks. It was presumably supplemented by the known hyperplastic effect of azoxymethane (Williamson et al, 1978d). The adaptive response to jejunal resection has been found to exceed that observed after jejunal bypass during the first postoperative week, but by 4 weeks the discrepancy has vanished

(Williamson et al, 1978a). Although adaptive changes in the small bowel persist for at least 3-6 months and probably indefinitely (Weser and Tawil, 1976; Williamson et al, 1978a; Williamson, 1982b), the lesser degrees of colonic adaptation have proved more difficult to detect at this stage using measurements of wet weight or crypt depth (Williamson et al, 1980b). In the present study the normal wet weight of the large intestine belied the apparent increase in crypt cell production rate 20-30 weeks after ileal resection or bypass.

Initially (at 20 weeks) ileal resection produced greater numbers of tumours than controls or rats with ileal bypass. These tumours were also larger, and they developed particularly in the proximal colon, where the maximal adaptive effect was observed. By 25 weeks ileal bypass also demonstrated a significant cocarcinogenic effect, whereas at 30 weeks the yield, size and distribution of tumours were similar in all three groups, although only a small number of rats in the resection group had survived to this stage. These findings suggest that a major factor in the promotion of neoplasia by postoperative hyperplasia is a reduction in the latent period between exposure to the carcinogen and tumour development (Hart et al, 1982). The first dose of carcinogen was given 1 week postoperatively, when the adaptive response to ileectomy would be clearly established, and the course was completed 5 weeks later, by which time the response to bypass would be expected to be of similar intensity.

EXPERIMENT B

Intestinal adaptation and carcinogenesis in defunctioned colon.

Background

In experiment A surgical shortening of the rat gut increased crypt cell proliferation in the remaining bowel and promoted colorectal carcinogenesis. As a corollary, environmental changes that depress cell turnover might be expected to decrease carcinogenesis in the affected bowel. Starvation and interruption of normal anatomical continuity are the two most effective methods of causing intestinal hypoplasia (Steiner et al, 1968; Terpstra et al, 1981). Individuals who are less than 80% of ideal body weight may enjoy some protection against colon cancer (Lew and Garfinkel, 1979). Defunctioning proximal colostomy greatly reduces the number of cancers in the distal colon of rats exposed to azoxymethane or dimethylhydrazine, agents that reach the mucosa largely via the bloodstream (Wittig et al, 1971; Campbell et al, 1975). With the contact carcinogen, 2,4-dimethyl-4-aminobiphenyl, which is delivered via the intestinal lumen, colostomy protects the distal colon completely (Cleveland et al, 1967; Navarrete and Spjut, 1967).

Not only the presence of faeces but its precise composition affect the development of colorectal neoplasia. Diets enriched with fat and depleted of fibre increase both faecal excretion of bile acids and the number of colonic bacteria capable of further degrading secondary bile acids to potential pre-carcinogens (Reddy and Wynder, 1973; Hill, 1974). Moreover, administration of cholic acid by mouth (Cohen et al, 1980) and of deoxycholic acid per rectum promote the development of experimental tumours (Reddy et al, 1976c,d).

This experiment used loops of rat colon isolated as a Thiry-Vella fistula (TVF) to examine the effect of defunction on the mucosal proliferative state and susceptibility to carcinogenesis and to compare the local effects of saline, deoxycholic acid and dilute faeces in altering the number of tumours induced by azoxymethane.

Design

One hundred and five rats weighing 120-150 g were allocated to 1 of 5 groups (Figure 10b:1). All animals received weekly intraperitoneal injections of azoxymethane 15 mg/kg for six weeks. Each rat was submitted to operation 7-12 days after the last injection of carcinogen.

At laparotomy the right colon was transected 1 cm distal to its origin from the caecum, and the left colon was transected just above the pelvic brim. In groups 2-5 the stump of right colon was anastomosed to the distal cut end of left colon, thus excluding the greater part of the large bowel from the faecal stream (Figure 10b:2b). This isolated loop was mobilised on the middle colic artery and its marginal branches and was brought to the surface at each end as a colonic Thiry-Vella fistula (TVF). Openings were created (5 mm diameter) in the muscle and skin of the abdominal wall on either side of the midline, and proximal and distal colostomies were established to drain the loop. In group 1 (sham TVF), normal colonic continuity was restored by re-uniting the colon at each point of transection (Figure 10b:2a). The proximal and distal stomas were secured with interrupted 6/0 chromic catgut sutures approximating mucosa to skin.

Rats in group 1 (sham TVF) and group 2 (TVF alone) had no further manipulations between operation and death. One week after operation the TVF was irrigated for the first time in groups 3-5 (Figure 10b:1) Irrigations

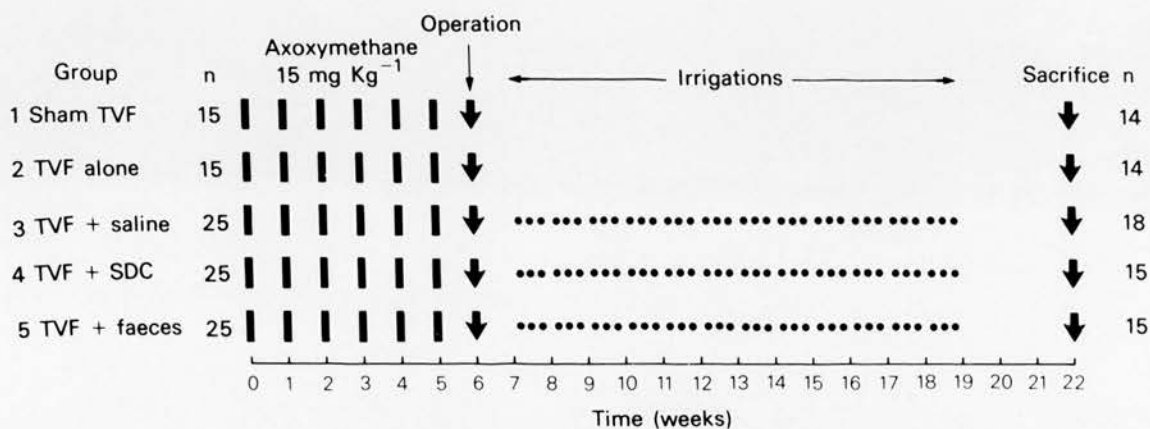


FIG. 10b:1 Experimental design. Number of animals at outset and surviving to the end of the experiment (>19 weeks) are shown. TVF = Thiry-Vella fistula: SDC = sodium deoxycholate.

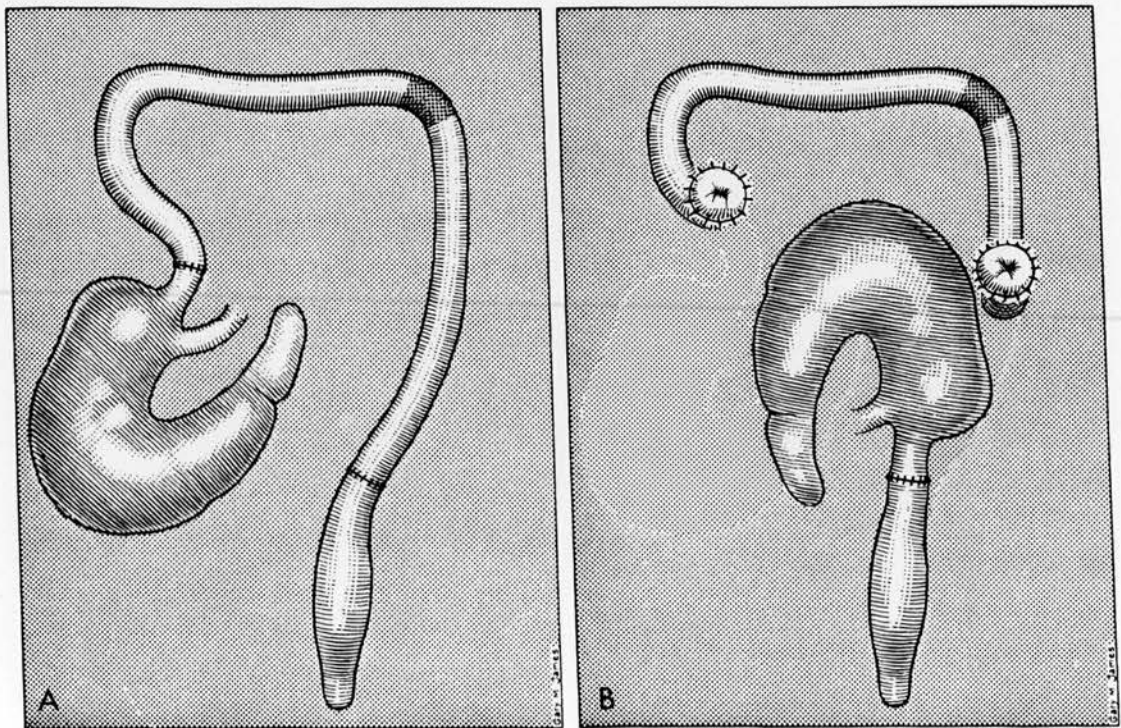


FIG. 10b:2 Operations performed. A = sham: B = Thiry-Vella Fistula. Shadow segments represent the sites of specimens taken for estimation of crypt depth.

were carried out using 5 ml plastic syringes, the hubs of which could be fitted comfortably into either stoma of rats suitably restrained by an experienced handler. An initial bolus of 5 ml N saline was administered to rats in all three groups to clear the fistula of mucus and retained faeces. It was not possible to carry out all irrigations from the same side. TVF contents (retained mucus and subsequently tumours) often produced a "ball valve" effect, making it necessary to instil small volumes from each end alternately.

Once patency had been demonstrated by the appearance of the irrigant at the opposite stoma, a second bolus of irrigant was administered. Rats in group 3 (TVF+saline) received a further 5 ml N saline. Group 4 rats (TVF+SDC) received 5 ml 0.12 M sodium deoxycholate, prepared by dissolving 50 g sodium deoxycholate (Sigma Chemical Co., St Louis, USA) in 1 litre N saline; each 5 ml aliquot contained 0.25 g (600 μ mol) Na deoxycholate. Group 5 rats (TVF+faeces) received 5 ml of a 12.5% w/v suspension of rat faeces in N saline prepared by collecting and homogenising faeces from rats not receiving carcinogen. The suspension was filtered through surgical gauze to remove the larger fibres and enable delivery into the fistulas via a syringe (Figure 10b:3). Each irrigant solution was administered three times a week (Monday, Wednesday, Friday) for 12 weeks, beginning at week 7 (Figure 10b:1).

Rats were killed when moribund or at the end of 22 weeks. At autopsy the following segments were thoroughly flushed with cold saline to remove all contents: duodenum, jejunioileum, caecum, colon between anastomoses (or TVF) and rectum. The length and wet weight of each segment were recorded, and the surface area of the caecum was estimated as previously described. The weights of the liver, kidneys and spleen were also recorded.

The number, size and position of all tumours were recorded.

A 1 cm specimen of colon was excised from the middle of each TVF or from the mid transverse colon in shams, and histological sections were prepared for estimation of mean crypt depth by ocular micrometry.

Results

Mortality rate

Nine rats (8.5%) died before the end of the first postoperative week from either haemorrhage or anaesthetic overdose. Most subsequent deaths resulted from rupture of the TVF during irrigation (9 rats), caecal volvulus around the TVF (5) or strangulated intestinal hernia (2). Three rats with suspected burst TVFs were re-explored and resutured immediately but could not be saved. The yields of surviving animals at the end of the experiment are given in Figure 10b:1).

Body weight

At the end of the first postoperative week the mean weight of the groups with a TVF was 5-24% lower than immediately before operation, while shams had regained their preoperative value. Thereafter, all rats gained weight steadily, but TVF rats remained a little lighter than the shams. At the end of the experiment, the mean weight of the TVF groups varied between 496 and 520 g, i.e. 91-96% of the weight of the shams (544 ± 16 g, SEM: $P < 0.05$).

Intestinal adaptation

The mean length of the TVF in all four groups was 12.1 ± 0.2 cm (SEM) and the mean weight was 2.1 ± 0.2 g. By contrast, the equivalent segment

of colon between the anastomoses in shams was 20.5 ± 0.6 cm long and weighed 3.8 ± 0.2 g ($P < 0.001$). Thus defunction as a TVF diminished the length and wet weight of the colon by 41 and 45% respectively. Among the four groups of TVF rats the mean TVF length ranged from 11.4-12.9 cm and the TVF weight from 1.7-2.9 g, irrespective of irrigation. No significant differences were found between any of the groups in the weight of the duodenum, jejunioileum, caecum, liver, kidneys or spleen, nor in the surface area of the caecum or the length of the other intestinal segments.

The mean colonic crypt depth (Figure 10b:3) in sham colons was $274 \mu\text{m} \pm 4$ (SEM), compared with 242 ± 6 in the non-irrigated TVFs and 241 ± 6 in the SDC-irrigated TVFs ($P < 0.001$). In those TVFs irrigated with faeces and saline, crypt depth did not differ from that in the shams (280 ± 6 and 276 ± 7).

Intestinal tumours

All but 2-3 rats in each group developed one or more tumours in the isolated colonic TVF (Figure 10b:4) or equivalent segment of functioning colon between anastomoses in the shams. The mean number of these tumours per rat in the four combined TVF groups (2.7) was 25% lower than the mean number in shams (3.6) (Table 10b:1). Including those tumours arising at a stoma or colonic anastomosis increased the numbers in both TVF rats (3.0) and shams (4.3) and increased the difference to 30% ($P < 0.05$). Tumour yields were lowest (64-67% of shams) in TVFs that were either not irrigated (TVF alone) or were irrigated with SDC ($P < 0.05$). In rats with saline or faecal irrigation yields were still only 79-85% of those in shams, but these differences no longer attained statistical significance.

Substantial numbers of tumours arose in the duodenum, jejunioileum

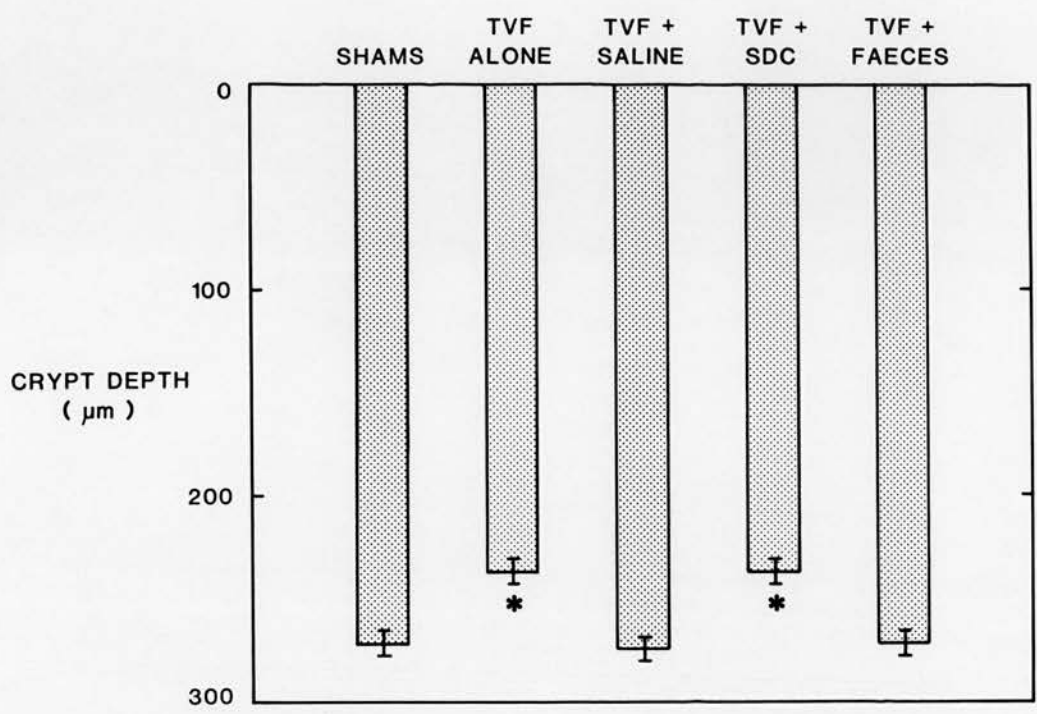


FIG. 10b:3 Colonic crypt depths (mean \pm s.e.m.)
TVF = Thiry-Vella fistula significance * $p < 0.001$ vs other three groups.



FIG. 10b:4 Tumour in Thiry-Vella fistula (TVF)
A = barium fistulogram B = same TVF opened at autopsy

	1 Sham TVF	2 TVF alone	3 TVF + saline	4 TVF + SDC	5 TVF + faeces	2-5 All TVFs
Duodenum	2.2 ± 0.4	2.2 ± 0.3	2.6 ± 0.5	2.1 ± 0.4	1.3 ± 0.4	2.1 ± 0.4
Jejunioileum	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.1
Colon (less stomas/anastomoses)	3.6 ± 0.5	2.4 ± 0.4*	2.9 ± 0.4	2.3 ± 0.4	3.1 ± 0.6	2.7 ± 0.2
Stomas/anastomoses	0.6 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
Total Colon	4.3 ± 0.6	2.6 ± 0.3*	3.2 ± 0.4	2.6 ± 0.4*	3.5 ± 0.6	3.0 ± 0.2*
Rectum (less anastomosis)	1.1 ± 0.4	1.9 ± 0.3	1.2 ± 0.3	0.9 ± 0.3	1.2 ± 0.3	1.3 ± 0.2
Caecorectal Anastomosis	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.1

Table 10:B1 Number of tumours per rat (mean ± SEM) in groups with and without a colonic TVF (Thiry-Vella fistula)

SDC = Sodium deoxycholate Significance versus shams * = $\bar{p} < 0.05$

and rectum in each group of animals, but these numbers were not affected by creation or irrigation of a colonic TVF (Table 10b:1). Tumours also arose on the rectal side of the colorectal anastomoses in shams and at the caecorectal anastomoses in TVFs. There were no differences in the yield of such suture-line tumours between the groups. In addition, 1 rat had a caecal tumour, 1 a gastric tumour and 5 had tumours of the external auditory canal. Carcinomatosis peritonei occurred in 11 rats.

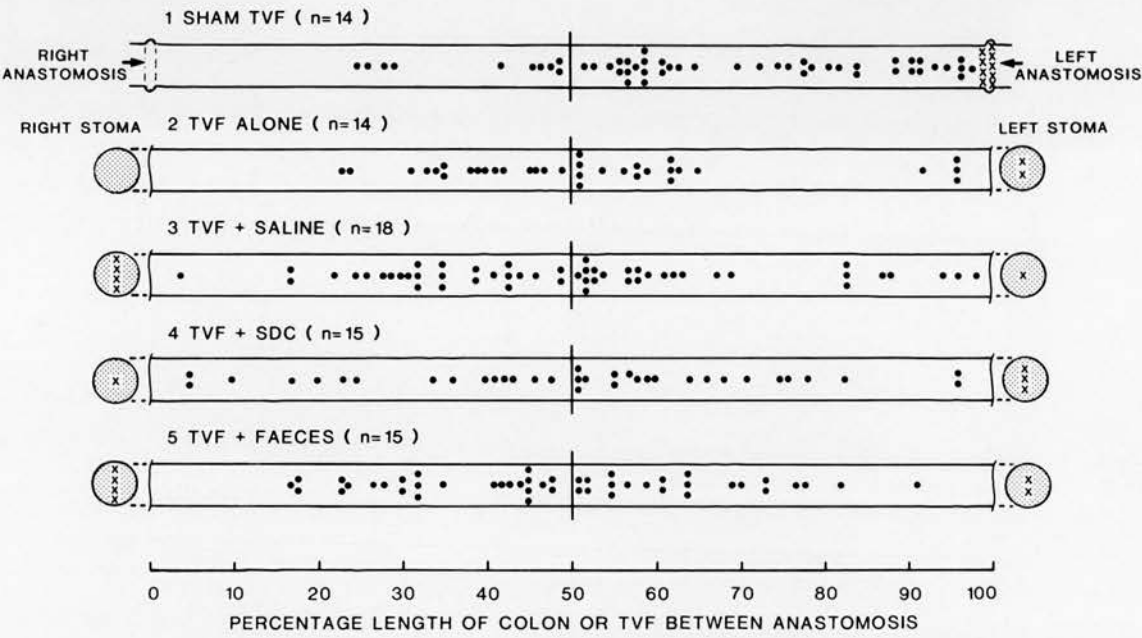
The presence of a TVF altered the distribution of colonic tumours irrespective of irrigation (Figure 10b:5). In sham rats 41 of 52 tumours (81%) arose within the distal half of the colon, whereas in TVF rats (groups 2-5) tumours were evenly distributed between the proximal (51%) and distal (49%) halves of the isolated colon. The yield of tumours in the proximal colon was 1.4 ± 0.1 in all TVFs compared with 0.7 ± 0.3 in the equivalent segment of the shams ($P < 0.05$). No differences were observed between the non-irrigated and irrigated TVFs. Similarly, in TVF rats, tumours were distributed almost equally between the right stoma (9 tumours) and the left stoma (8), while in shams anastomotic tumours (9) were confined to the distal colon.

The mean diameter of tumours found in the TVFs irrigated with sodium deoxycholate (2.6 ± 0.3 mm) was 41-51% less than the diameter of colonic tumours in the other four groups ($P < 0.01$). There were no other significant differences, however, and creating a TVF alone did not reduce tumour size (Figure 10b:6).

Comment

Colon excluded from the faecal stream develops fewer tumours than colon remaining in continuity. This finding can hardly be explained by the

FIG.10b:5



DISTRIBUTION OF TUMOUR IN THIRY-VELLA FISTULAS (TVF) OR SHAM TVF.
EACH TUMOUR IN THE TVF (IN EQUIVALENT COLON) IS SHOWN BY A SOLID CIRCLE.
EACH TUMOUR AT A STOMA OR ANASTAMOSIS IS SHOWN BY A CROSS.

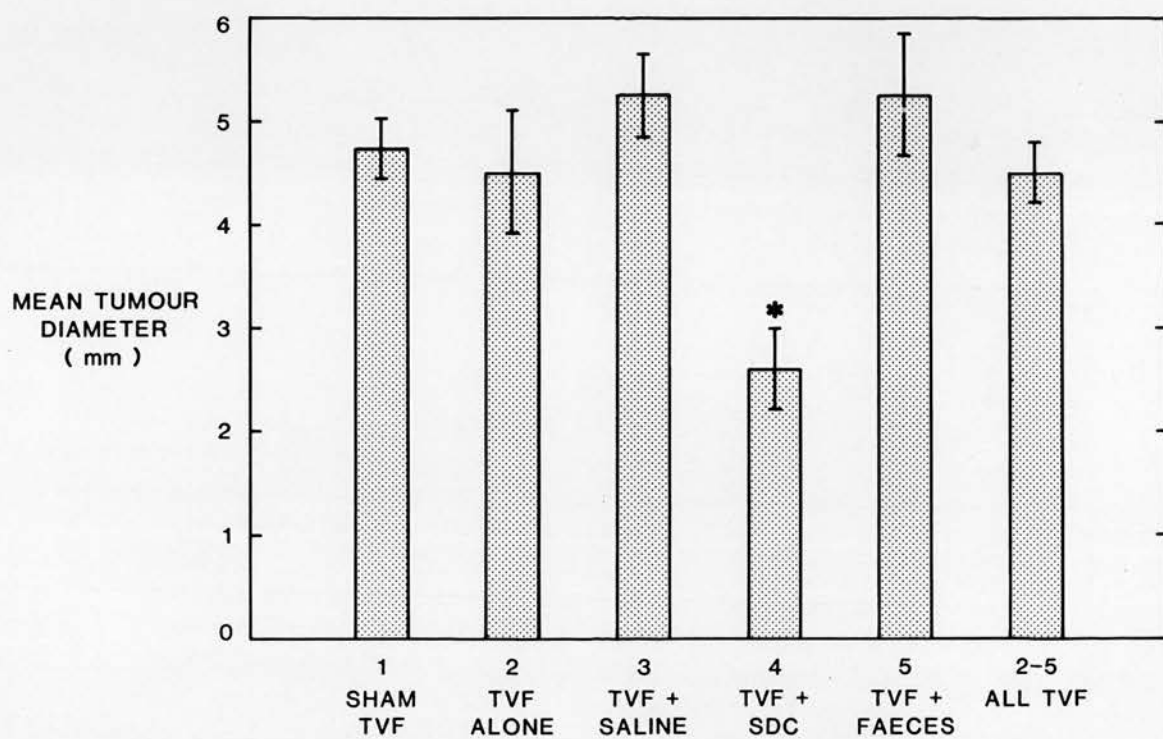


FIG. 10b:6 Mean tumour diameter. TVF = Thiry-Vella fistula.
SDC = sodium deoxycholate. Significance: * $p < 0.05$ vs all other groups.

minor decrease in body weight found in the TVF rats, since 85% jejunoileal bypass has been found to enhance colonic carcinogenesis despite a 40% reduction in body weight (Bristol et al, 1984). The results are consistent with the findings of other workers (Wittig et al, 1971; Campbell et al, 1975), who have ascribed the protective effect of a defunctioning proximal colostomy to an altered population of bile acids and bacteria in the isolated distal colonic segment. As neither resection nor bypass of the small intestine affect carcinogenesis in bypassed colon, hormones appear to be of secondary importance to diversion of the faecal stream (Bristol et al, 1982a). Rubio and colleagues in a similar experiment found no reduction in dimethylhydrazine-induced tumours in a small colonic TVF, but the number of rats was small and no control group was included (Rubio et al, 1980).

Mucosal hypoplasia in the bypassed colon is indicated by shortening of the crypts and by the reduced weights and lengths of the TVFs; presumably this phenomenon accounts for the reduced susceptibility to carcinogenesis. There was a close correlation between tumour yield and crypt depth, each being reduced in the TVF (with or without deoxycholate) but indistinguishable from values in intact colon after irrigation of the TVF with saline or faeces. Other studies confirm that mucosal atrophy develops rapidly distal to the site of a proximal colostomy but is precisely reversed soon after continuity of the bowel is restored (Tilson et al, 1976; Terpstra et al, 1981).

These results may shed some light on the role of various faecal constituents in colorectal carcinogenesis. Clearly, deoxycholate was not cocarcinogenic in this model, despite strong epidemiological and experimental evidence to support such a role for bile acids (Chapter 8). Introduction of a secondary bile acid into isolated colon promoted neither

hyperplasia nor neoplasia. Indeed deoxycholate irrigation actually reduced tumour size suggesting a possible protective effect. These findings are at variance with the studies of Reddy and his colleagues showing enhanced carcinogenesis after the instillation of primary and secondary bile acids into the intact rectum of conventional and germ-free rats. There are certain methodological differences. In the experiment (Narisawa et al, 1974) the bile acid was suspended in peanut oil, which might itself be carcinogenic, and appropriate controls were not included. In two others (Reddy et al, 1976c, 1977b) a smaller total dose of deoxycholate was used (3 g versus 9 g), and in all three the direct-acting carcinogen N-methyl-N'-nitro-N-nitrosoguanidine was employed rather than parenteral dimethylhydrazine or azoxymethane which require metabolic activation. No doubt the intestinal microflora, implicated in the cocarcinogenic role of bile acids, are both quantitatively and qualitatively different in a TVF as opposed to colorectum in continuity. Moreover, the absence of faeces might remove some constituent that is necessary for bile acids to exert their promoting effect or is itself an additional cocarcinogen. Ammonia and other products of protein and urea degradation have been suggested for this role (Wynder and Reddy, 1973), and the mechanical stimulus of faecal bulk could also be important.

Deoxycholate irrigation alone did not prevent the reduction in crypt depth found in the non-irrigated TVFs. Experiments showing that bile and pancreatic juice could stimulate adaptive growth and carcinogenesis after surgical diversion involved distal gut that remained in continuity with the faecal stream (Williamson et al, 1978c, 1979). Bile acid solutions are detergents, and this property could outweigh any cocarcinogenic role by cleansing the fistula of accumulated cellular debris and retained faeces that

saline failed to dislodge.

Tumour yields in the TVFs irrigated with faeces were greater than in the non-irrigated TVFs but did not quite reach the level found in the shams. Faecal bulk is important in the maintenance of normal cell turnover (Williamson, 1982b) and was clearly diminished by the 8-fold dilution required to permit its delivery into the fistulas. The faecal irrigant failed to prevent the loss of weight and length in the TVF, but it did preserve normal crypt depth and it enhanced carcinogenesis. Mechanical stimulation may be important, since saline irrigation had similar effects. Clarke has shown that saline stimulates mucosal cell turnover in isolated loops of small bowel (Clarke, 1977). The relative lack of bulk in the irrigant may have facilitated cocarcinogenic activity in other faecal constituents, since it has been suggested that the bulking action of dietary fibre has a protective effect in human large-bowel cancer by reducing exposure of the colonic mucosa to putative faecal carcinogens (Heaton, 1977).

The anatomical redistribution of tumours in the TVF (regardless of irrigation) is of great interest. As the proximal colonic tumour yield was actually increased in the sequestered colon, the redistribution observed was not just the result of a relative reduction in the proportion of distal colonic tumours, which has been observed in low incidence populations and in animals receiving low-dose carcinogen (Lambert, 1982; Ross, 1982). The various substances administered did not affect the yield of proximal tumours. Since irrigation was not confined to one or other stoma, it is unlikely that the altered distribution of tumours was due to any "jet" effect. The normal left-sided preponderance of colonic tumours could reflect differences between the proximal and distal colon in the bulk and transit time of faeces, the population and activity of bacteria, and the rate of

mucosal cell proliferation (Cooke et al, 1982). Withdrawal of faeces would change all these conditions.

Several tumours were observed at stomas. Colostomy tumours in rats given azoxymethane have been reported (Terpstra et al, 1981). Stomal cancers can develop spontaneously in Wistar rats not receiving carcinogen, probably owing to chronic irritation (Winkler, 1982). Similar susceptibilities of the ascending and descending colostomies mirror the redistribution seen within the TVF and contrast with the finding in shams, in which anastomotic tumours were confined to the left colon.

The failure of colonic bypass to increase carcinogenesis in the adjacent gut is not surprising, at least in the case of the ileum which is extremely resistant to cancer (Williamson, 1982a). Although subtotal colectomy including caecectomy has a mild enhancing effect on rectal carcinogenesis, hemicolectomy has no such effect (Williamson et al, 1980b, 1982). The caecum was retained in continuity in this experiment, and this may have prevented any promotion of rectal carcinogenesis. Moreover bypass is probably a less potent promoter of carcinogenesis than resection (Williamson et al, 1980b; Experiment A).

EXPERIMENT C

Mucosal cell proliferation in defunctioned colon and bowel remaining in continuity

Background

In experiment B isolating a long segment of colon from the faecal stream as a Thiry-Vella fistula (TVF) depressed carcinogenesis within it. This manoeuvre also produced significant reductions in the length, wet weight and mean crypt depths of the defunctioned segment. Although based on simple measurements, these findings support the contention that exclusion from the faecal stream results in colonic hypoplasia (Tilson et al, 1976; Terpstra et al, 1981).

Irrigation of TVFs with saline and faeces (but not deoxycholate) restored both crypt depth and tumour yield to levels found in colon-in-continuity. This close correlation between crypt depth and carcinogenesis suggests that the reduced susceptibility of defunctioned colon to carcinogenesis is directly linked to mucosal hypoplasia.

The crypt cell production rate (CCPR) is held to be a more sensitive index of the mucosal proliferative state than the simple morphometric measurement of crypt depth (Chapter 9). In this experiment the CCPR was determined in normal colon-in-continuity (sham TVF), in defunctioned colon (TVF) and in adjacent intestinal segments 1, 4 and 16 weeks after surgery.

Azoxymethane may have a significant and prolonged hyperplastic effect on intestinal mucosa (Williamson et al, 1978d) (Experiment A). This postulate was explored in normal and defunctioned colon and adjacent segments at 4 and 16 weeks postoperatively.

Design

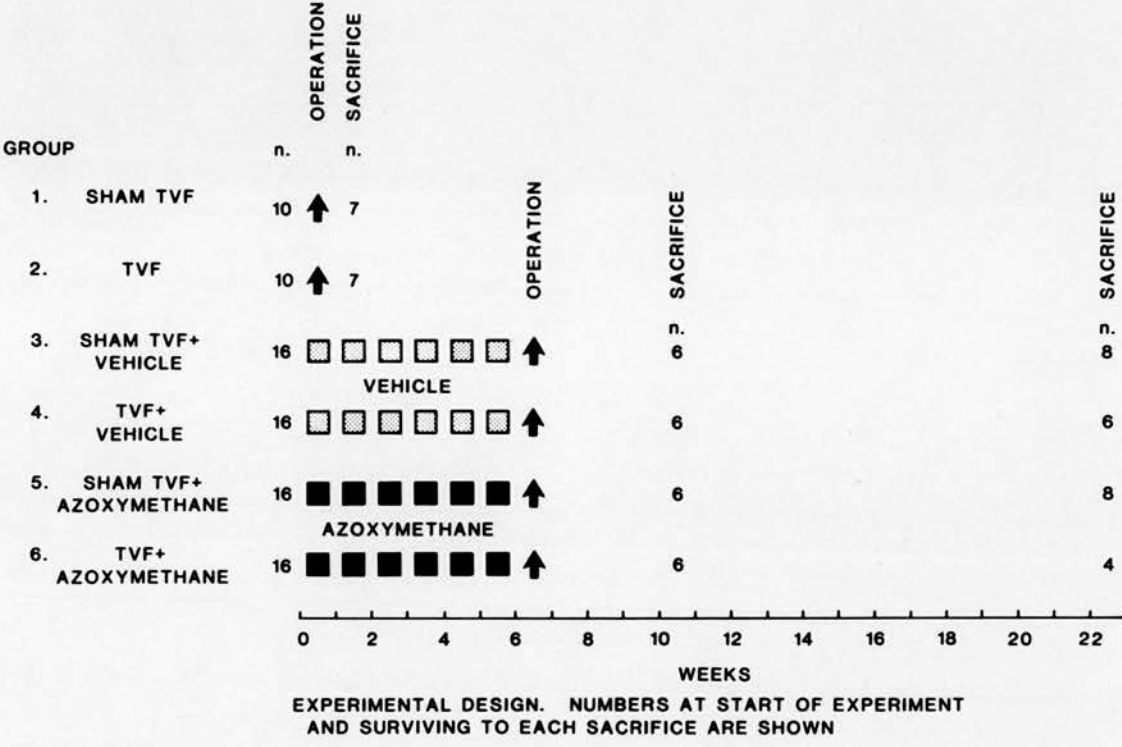
Eighty-four rats were allocated to six groups (1-6; Figure 10c:1). Animals in groups 1 and 2 were from a different batch and weighed more at the start of the experiment (290-320 g) than those in groups 3-6 (190-230 g); they underwent operation during week 1. In group 2 (n=10) Thirty Vella fistulas (TVFs) were fashioned as described in Experiment B (Figure 10b:2b) and the equivalent sham TVF operation was carried out in group 1 (n=10; Figure 10b:2a). None of these rats received carcinogen and they were all killed on the 7th postoperative day.

Groups 3-6, each comprising 16 rats, received a 6-week course of subcutaneous injections before surgery in week 7. Groups 5 and 6 received azoxymethane 15 mg/kg weekly, while groups 3 and 4 received equivalent volumes of sterile water (vehicle). TVFs were raised in groups 4 and 6, and the sham procedure was performed in groups 3 and 5 (Figure 10c:1).

Six rats from groups 3-6 were sacrificed 4 weeks after operation (week 11), and the survivors were killed 12 weeks later (week 23) (Figure 10c:1). No irrigations of TVFs were carried out in this experiment. At autopsy, the following segments were flushed clean with saline: duodenum, jejunoileum, TVF or colon between anastomosis, and rectum. Their length and wet weight (after excision of tumours in groups 5 and 6) were recorded. The weights of the caecum, liver, kidneys and spleen were also noted.

At the various times of sacrifice (1, 4 and 16 weeks after surgery), 6 rats in each group received an intraperitoneal injection of vincristine (1 mg/kg) and were sacrificed at half-hourly intervals from 30-180 minutes later. Specimens from these animals were processed for estimation of the crypt cell production rate as described in Chapter 9. The following segments were examined: terminal ileum, caecum, right, middle and left

FIG.10c:1



colon and rectum. The colonic segments comprised the isolated TVF or large bowel between the anastomoses in shams.

Results

Mortality

Numbers surviving to sacrifice in each group are given in Figure 10c:1). There were 14 deaths during the first postoperative week from anaesthetic overdose (7), haemorrhage (3), anastomotic leakage (2), small-bowel infarction (1) and obstruction (1). Six rats died during the last month of the experiment: 3 from intestinal cancer and 1 from carcinoma of the external auditory meatus. In 2 rats the cause of death could not be determined. All rats dying of cancer had received azoxymethane.

Body weight

During the first postoperative week rats in groups 1 and 2 lost weight (Figure 10c:2). Mean body weights were lowest on the 3rd and 4th days: 9% and 13% lower than preoperative values in group 1 (sham TVF) and 2 (TVF) respectively. Towards the end of the week rats in both groups were regaining weight but the mean body weight in group 1 (290 ± 25 g: \pm s.d.) and group 2 (282 ± 25) remained 4% and 8% lower than their respective preoperative values (Figure 10c:2).

Groups 3-6 showed a similar fall in the first postoperative week which was restored within 3 weeks but gained weight steadily thereafter (Figure 10c:3). Throughout the postoperative phase the mean body weight in group 3 (sham TVF + vehicle) remained consistently higher than that of the other groups, but this difference did not attain statistical significance (Figure 10c:3).

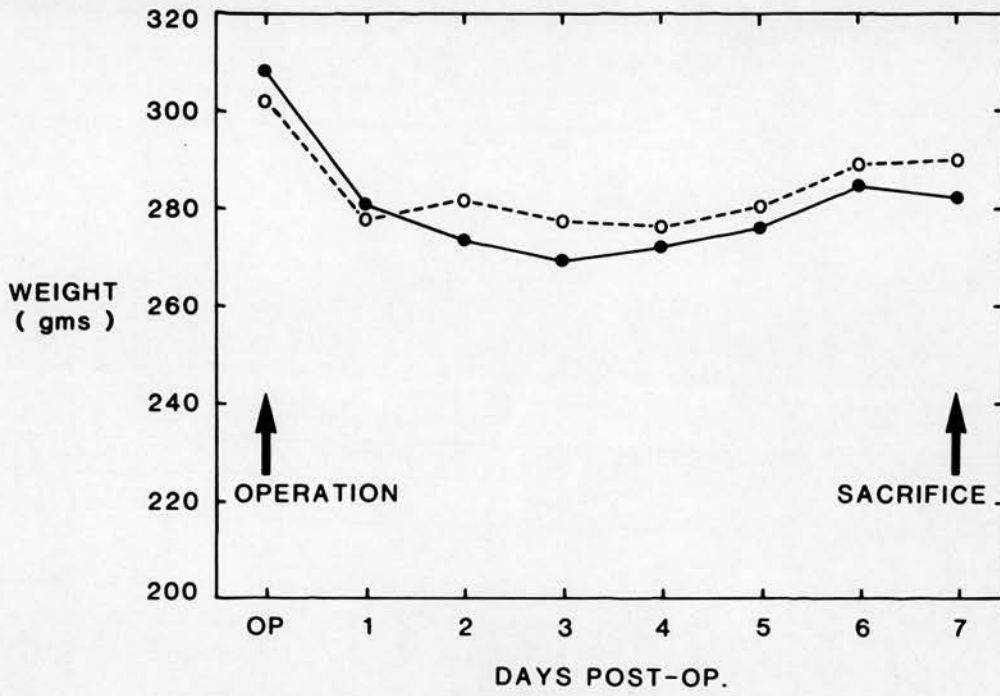


FIG.10c:2 MEAN BODY WEIGHT DAILY FROM OPERATION (DAY 0)
TO SACRIFICE (DAY 7)

o---o GROUP 1 SHAM TVF

●---● GROUP 2 TVF

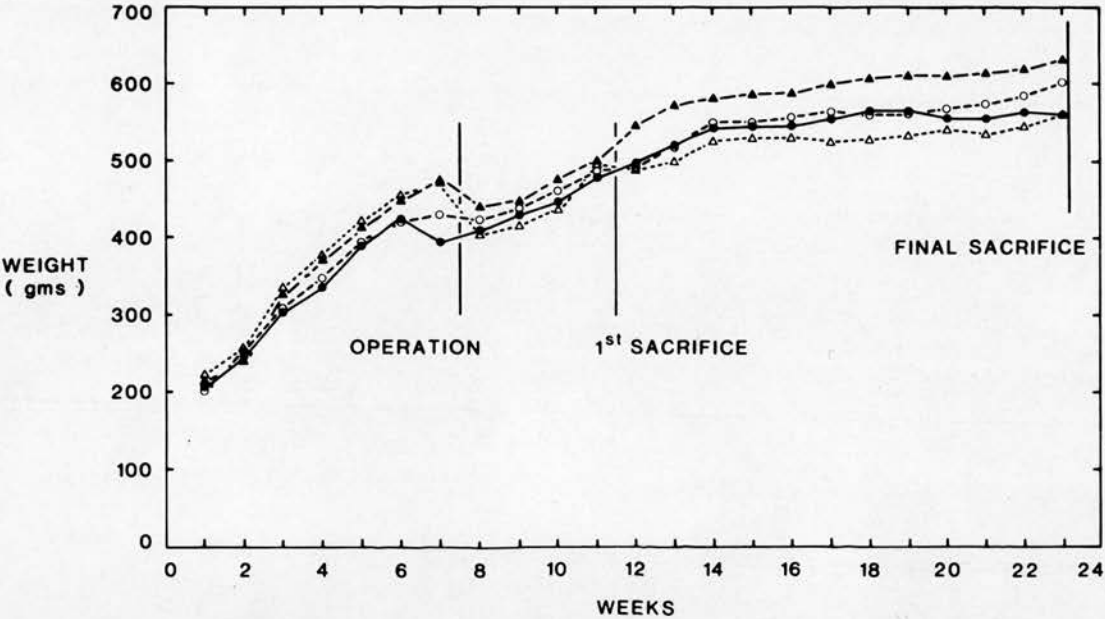


FIG.10c:3 WEEKLY MEAN BODY WEIGHT (gms) IN GROUPS 3-6

●—● GROUP 6 TVF-AOM ○—○ GROUP 5 SHAM-AOM △—△ GROUP 4 TVF-VEHICLE
▲—▲ GROUP 3 SHAM-VEHICLE

Intestinal adaptation

After one week the isolated colonic loops (TVFs) were slightly shorter and weighed 25% less than the equivalent segment in the shams, but these differences were not statistically significant (Table 10c:1). Again at 4 weeks there was a similar trend for the TVFs (groups 4 and 6) to be shorter and weigh less than the equivalent segments of colon-in-continuity (group 3 and 5), irrespective of azoxymethane administration (Table 10c:2). By 16 weeks the isolated TVFs in groups 4 and 6 weighed 42-45% less ($P < 0.02$) and were 28-39% shorter ($P < 0.001$) than the equivalent segments in the sham groups 3 and 5 (Table 10c:3).

Surprisingly, the caecum was consistently heavier (9-72%) in the sham groups than in the TVF groups at each time of sacrifice although the differences only reached statistical significance ($P < 0.05$) between groups 3 and 4 at 4 weeks (Tables 10c:1,2,3). No differences between the groups were detected in the weights and lengths of the other segments or in the weights of the liver, kidneys or spleen at any of the sacrifices.

Crypt cell production rates

The raising of a TVF produced increases in the CCPR of 63% in the terminal ileum, 122% in the caecum and 119% in the rectum at one week, which did not reach statistical significance (Figure 10c:4a). Crypt cell production rates were not reduced in the TVF itself. Indeed there was an apparent 53% increase in the mid colon.

At 4 weeks in the groups not receiving carcinogen (3,4) the CCPR in the ileum was 167% higher after TVF than in the shams ($P < 0.01$) and the rectal CCPR was increased by 86% ($P < 0.05$) (Figure 10c:4b). There was no difference between groups in the CCPR in the caecum or in the mid and left

		Group 1 Sham TVF	Group 2 TVF
Body Weight		290.4 \pm 24.5	282.1 \pm 25.4
Duodenum	Length	10.0 \pm 0.4	10.3 \pm 1.0
	Weight	0.9 \pm 0.1	1.8 \pm 0.2
Jejunioileum	Length	117.7 \pm 6.6	120.0 \pm 7.0
	Weight	6.6 \pm 1.5	5.3 \pm 1.1
Caecum	Weight	3.5 \pm 1.4	2.4 \pm 0.6
Colon (TVF or sham)	Length	13.7 \pm 2.1	12.1 \pm 1.2
	Weight	1.6 \pm 0.4	1.5 \pm 0.2
Rectum	Length	6.6 \pm 1.1	7.5 \pm 1.0
	Weight	0.8 \pm 0.2	0.5 \pm 0.2

Table 10c:1

Body weight (g) and lengths (cm) and weights (g) of intestinal segments in groups 1 and 2 (mean \pm S.D.), at sacrifice (1 week).

		Group 3 Sham TVF + Vehicle		Group 4 TVF + Vehicle		Group 5 Sham TVF + AOM		Group 6 TVF + AOM	
Body Weight		459.2 \pm 68.6		499.2 \pm 38.6		475.3 \pm 48.4		455.0 \pm 60.7	
Duodenum	Length	12.2 \pm 1.2		11.8 \pm 1.1		11.4 \pm 0.9		10.3 \pm 0.9	
	Weight	1.5 \pm 0.4		1.4 \pm 0.2		1.1 \pm 0.2		1.1 \pm 0.2	
Jejunioileum	Length	129.2 \pm 13.1		132.0 \pm 4.3		139.5 \pm 11.6		137.7 \pm 12.3	
	Weight	11.3 \pm 2.3		11.5 \pm 1.5		10.0 \pm 1.7		8.9 \pm 4.4	
Caecum	Weight	6.2 \pm 1.1		3.6 \pm 1.8*		4.1 \pm 2.3		3.1 \pm 1.0	
Colon (TVF or sham)	Length	13.7 \pm 2.4		11.2 \pm 1.2		14.7 \pm 2.7		12.6 \pm 1.2	
	Weight	1.7 \pm 0.3		1.5 \pm 0.2		1.5 \pm 0.5		1.0 \pm 0.2**	
Rectum	Length	8.4 \pm 0.6		8.1 \pm 1.6		9.1 \pm 0.7		8.3 \pm 0.8	
	Weight	1.0 \pm 0.2		1.2 \pm 0.3		0.9 \pm 0.2		1.0 \pm 0.1	

Table 10c:2 Body weight (g) and lengths (cm) and weights (g) of intestinal segments (mean \pm S.D.) in rats in groups 3-6 sacrificed 4 weeks postoperatively.

* $P < 0.05$ vs grp 3 ** $P < 0.05$ vs grp 5

		Group 3 Sham TVF + Vehicle	Group 4 TVF + Vehicle	Group 5 Sham TVF + AOM	Group 6 TVF + AOM
Body Weight		632.3 \pm 61.1	562.0 \pm 59.2	600.8 \pm 84.3	561.5 \pm 72.9
Duodenum	Length	13.4 \pm 0.9	11.4 \pm 1.7	124.0 \pm 1.7	12.4 \pm 1.5
	Weight	1.2 \pm 0.1	1.0 \pm 0.3	1.5 \pm 0.4	1.5 \pm 0.5
Jejunioileum	Length	141.8 \pm 10.1	133.1 \pm 5.6	135.0 \pm 5.3	143.5 \pm 8.9
	Weight	10.7 \pm 1.1	10.1 \pm 0.9	12.2 \pm 4.7	9.7 \pm 1.3
Caecum	Weight	3.7 \pm 1.1	3.4 \pm 0.6	5.5 \pm 4.6	3.0 \pm 0.7
Colon (TVF or sham)	Length	17.7 \pm 2.0	10.8 \pm 0.9**	16.6 \pm 1.6	11.9 \pm 0.5**
	Weight	1.9 \pm 0.3	1.1 \pm 0.5*	2.0 \pm 0.5	1.1 \pm 0.3*
Rectum	Length	9.7 \pm 1.3	8.2 \pm 1.6	8.3 \pm 1.3	7.9 \pm 0.2
	Weight	1.0 \pm 0.1	0.9 \pm 0.2	0.9 \pm 0.3	0.9 \pm 0.1

Table 10c:3 Body weight (g) and lengths (cm) and weights (g) of intestinal segments (mean \pm S.D.) in rats in groups 3-6 sacrificed 16 weeks postoperatively.

* $P < 0.02$ vs grp 3 ** $P < 0.001$ vs grp 3 * $P < 0.02$ vs grp 5
 ** $P < 0.001$ vs grp 5

colonic segments (TVF or colon-in-continuity). There was, however, a suggestion of hypoplasia in the proximal part of the TVF (right colon); here the CCPR was less than half that of the same segment in continuity ($0.1 > \underline{P} > 0.05$) (Figure 10c:4b).

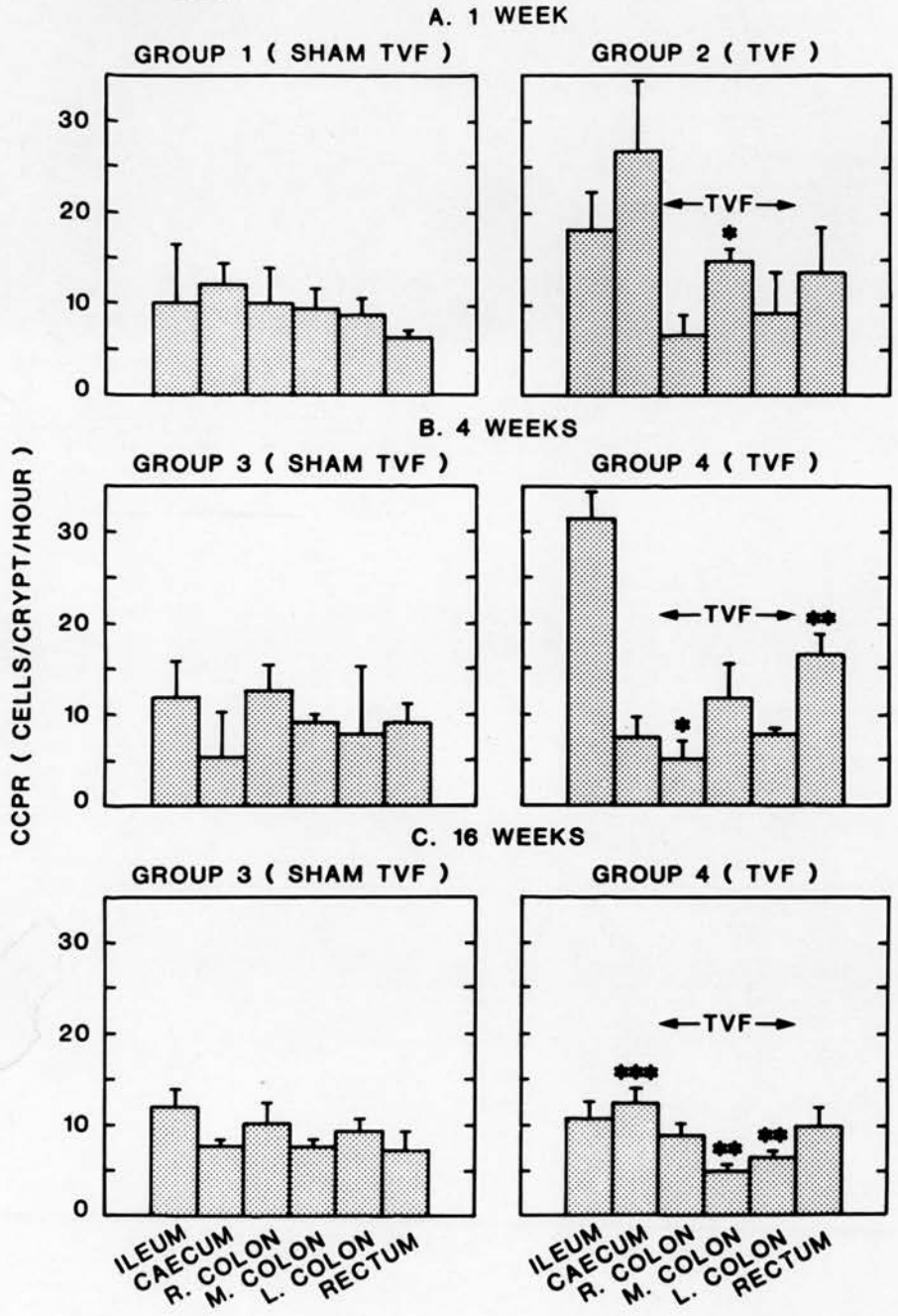
In the same two groups the hyperplasia found in the ileum and rectum had disappeared by 16 weeks (Figure 10c:4a), but caecal CCPR was 59% higher in the TVF group ($\underline{P} < 0.01$). The hypoplasia observed in the right colonic segment of the TVF at 4 weeks was now less evident and replaced by reductions in CCPR in the mid colonic (21%) and left colonic (32%) segments ($\underline{P} < 0.05$) (Figure 10c:4b).

At 4 weeks no significant differences in CCPR were found between the sham and TVF groups receiving azoxymethane (Figure 10c:5a). Rates in the ileum, caecum and rectum were only marginally higher after TVF and there was no evidence of hypoplasia in the defunctioned colon. This pattern was repeated at 16 weeks (Figure 10c:5b). The increased CCPR found in the caecum of TVF rats not receiving carcinogen (group 4) was also present in those receiving azoxymethane (group 6) but was matched by a similar increase in the azoxymethane-sham group (5) (Figure 10c:5b). Reductions in the CCPR found in the middle and left colonic segments of the non-azoxymethane TVFs were absent in the azoxymethane-treated TVFs.

Intestinal tumours

Tumours developed only in azoxymethane-treated rats (groups 5 and 6) (Figure 10c:6). The small numbers of rats surviving to sacrifice in these groups rendered statistical analysis of the data unhelpful, but the yield of colonic tumours in the TVFs (1.3 ± 0.6 mean \pm SEM) was only a third of that in the sham colons (3.8 ± 0.8). There were no differences between the

FIG.10c:4

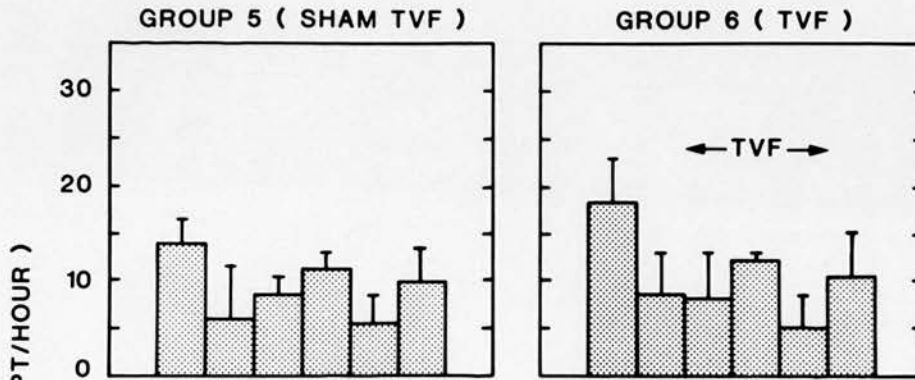


CRYPT CELL PRODUCTION RATES (CCPR=CELLS/CRYPT/HOUR \pm S.E.)
 IN INTESTINAL SEGMENTS AT 1 WEEK (A), 4 WEEKS (B) AND 16 WEEKS (C)
 POSTOPERATIVELY IN RATS NOT RECEIVING CARCINOGEN

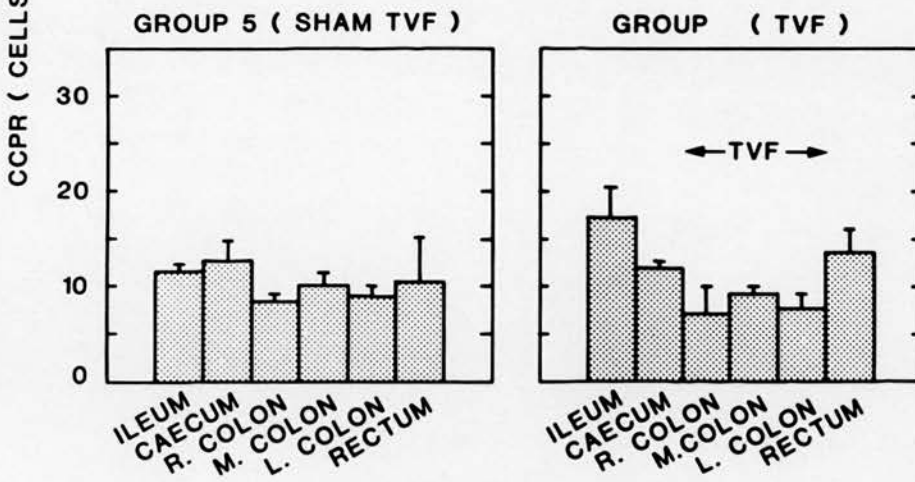
*0.1<P<0.05 **P<0.05 ***P<0.001 vs SHAMS

FIG.10c:5

A. 4 WEEKS



B. 16 WEEKS



CRYPT CELL PRODUCTION RATES (CCPR=CELLS/CRYPT/HOUR \pm S.E.)
 IN INTESTINAL SEGMENTS 4 WEEKS (A) AND 16 WEEKS (B)
 POSTOPERATIVELY IN RATS RECEIVING AZOXYMETHANE

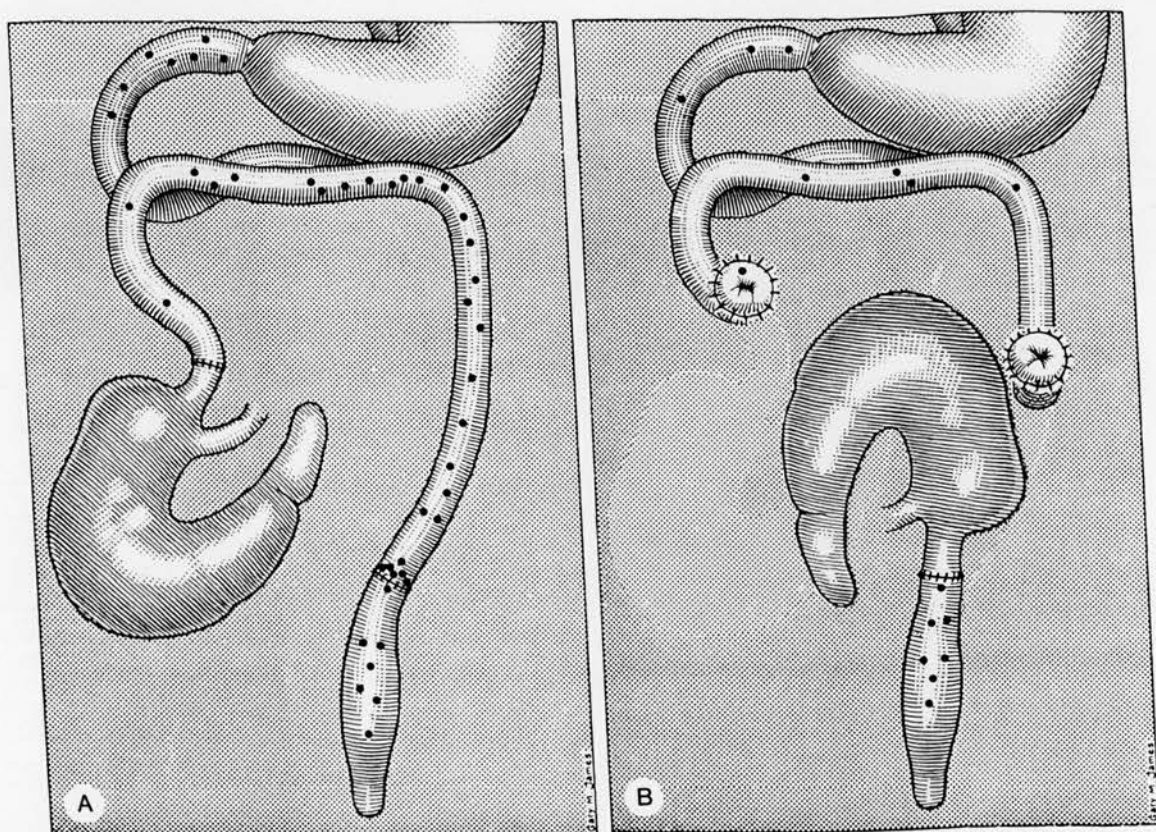


FIG. 10c:6 Distribution of tumours in group 5 (sham TVF + azoxymethane: $n = 8$) and group 6 (TVF + azoxymethane: $n = 4$)

groups in the yield of duodenal and rectal tumours. There were no jejunoileal tumours in either group and 1 rat in group 6 (TVF) developed a tumour of the external auditory canal.

Comment

This study confirms that isolating a segment of large bowel as a Thiry-Vella fistula results in significant reductions in its length and weight (Experiment B). Evidence of this progressive atrophy was seen as early as 1 week after operation, was more obvious at 4 weeks and was clearcut by 16 weeks.

The CCPR data were, as in Experiment A, generally disappointing. Several apparently major differences between groups did not stand up to statistical analysis. However, certain trends did emerge. Creating a TVF (effectively a colonic bypass) produces hyperplasia in bowel remaining in continuity. This phenomenon has been observed previously following small bowel resection and bypass (Experiment A) (Oscarson et al, 1979; Williamson et al, 1978a,b, 1982), where hyperplasia is generally maximal in bowel adjacent to the resected or bypassed segment. In the present study, however, the caecum was initially slow to respond, but the ileum, although further from the resection-line, showed significant hyperplasia by 4 weeks. Caecal hyperplasia became apparent at 16 weeks, by which time ileal hyperplasia had waned. Interestingly, caecal weight was consistently less after TVF than in the shams despite moderate increases in CCPR. This apparent paradox is difficult to explain. Fashioning a TVF might result in denervation of the caecum, with a consequent alteration of its motility, so that it becomes merely a conduit for bowel content rather than a reservoir and mill. The caecal muscle coat may therefore atrophy, while the mucosa

still responds to systemic factors mediating the adaptive response to intestinal bypass.

Even at its maximum, the caecal increment in CCPR (59%) was modest compared to the maximal ileal response (167%). The small bowel appears generally more sensitive to the adaptive effects of intestinal resection and bypass than the large bowel (Williamson, 1978). Nevertheless, in the rectum immediately distal to the resection line the CCPR increased rapidly after creating a TVF. This increase (86%) was less than that in the ileum with a peak at 4 weeks and tailing off thereafter. Other workers have found evidence of rectal adaptation to loss of functioning colon (Owen and Lyttle, 1979; Williamson et al, 1980b). It is interesting that none of the CCPR increases in segments remaining in continuity were paralleled by measurable differences in their length and wet weight. By contrast the clear reductions of length and weight of the TVFs were associated with relatively modest reductions in CCPR. Perhaps the crypt cell production rate is a more sensitive index of hyperplasia than hypoplasia. Evidence of hypoplasia appeared earlier in the right colon than in the other two defunctioned segments. The CCPR in the mid colon was actually increased 1 week and 4 weeks after defunction, owing perhaps to local hyperaemia; the middle colic artery is initially the sole source of blood to the fistula, though later the stoma and adjacent colon could be supplied by vessels in the abdominal wall.

In Experiment B, colonic atrophy found in the TVFs (evidenced by decreased weight, length and crypt depth) was implicated in their reduced susceptibility to azoxymethane-induced carcinogenesis. The CCPR data in this study confirm that defunctioned colon develops progressive mucosal hypoplasia in rats not receiving carcinogen. However, these findings did not

appear to hold true for rats receiving azoxymethane. Overall, CCPRs were not higher in the azoxymethane groups than in the non-azoxymethane groups but both the hyperplasia in segments remaining in continuity and the hypoplasia in defunctioned segments were less clear-cut and in some cases undetectable. Azoxymethane may have a long-term stimulatory effect on intestinal mucosa (Experiment A), which in this study masks the reductions in CCPR found in the TVFs of rats not receiving carcinogen. Yet colonic defunction does partly protect against carcinogenesis (Experiment B). Although this study was not designed primarily to re-examine carcinogenesis in defunctioned colon, tumours occurred three times more frequently in colon-in-continuity than in defunctioned TVFS.

EXPERIMENT D

Colorectal carcinogenesis after intrarectal instillation of a bile acid with and without concurrent oral metronidazole

Background

Bile acids may be endogenous cocarcinogens in colorectal cancer (Reddy, 1981; Thompson, 1982) (Chapter 8). They could exert a direct mutagenic effect on the epithelial cell or act indirectly by altering the rate of mucosal cell proliferation and hence susceptibility to carcinogenesis. In the rat, diversion of pancreaticobiliary secretions to mid-small bowel enhances colorectal carcinogenesis (Chomchai et al, 1974; Williamson et al, 1979) and also stimulates marked adaptive hyperplasia of the ileum and moderate colonic hyperplasia (Williamson et al, 1978c). However, bile acids appear to be neither tropic nor cocarcinogenic to hypoplastic defunctioned colon (Experiment B).

This experiment was designed to test the tropic and cocarcinogenic potential of sodium deoxycholate instilled directly into the large bowel of rats exposed to azoxymethane. The composition of the colonic microbial flora is implicated as the key intermediary modulating the effect of luminal bile acids (Aries et al, 1969; Hill, 1979). Anaerobes, in particular, may metabolise bile acids to yield products which are carcinogenic or cocarcinogenic (Hill, 1974). Therefore, the effect of oral metronidazole in modifying this potential was examined.

Design

One hundred and fifteen rats weighing 70-100 g were allocated to one of five groups (Figure 10d:1). All animals received weekly subcutaneous

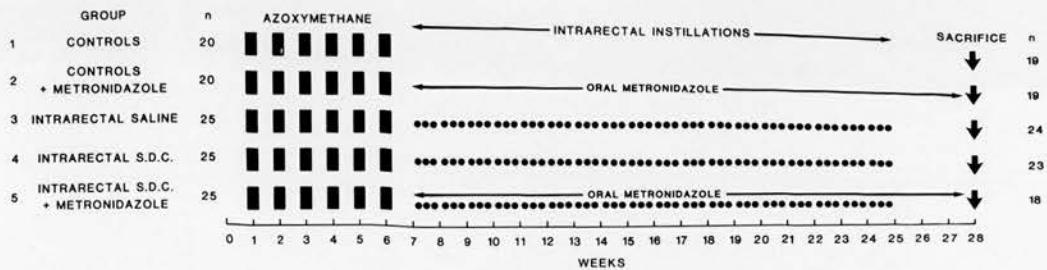


FIG. 10d:1 Experimental design: SDC = sodium deoxycholate. Numbers in each group at the outset and surviving until sacrifice are shown.



FIG. 10d:2 Tumours in distal colorectum (the cannula was inserted through the anus to a distance of 5 cm).

injections of azoxymethane 15 mg/kg for 6 weeks (Figure 10d:1).

One week after the last injection of azoxymethane, intrarectal instillations were carried out for the first time in groups 3-5. Colonic wash-out was not carried out and anaesthesia was unnecessary. An 18 gauge plastic intravenous cannula was inserted through the anus to a distance of 5 cm in rats suitably restrained by an experienced handler (Figure 10d:2). Rats in group 3 received 1 ml N saline. Groups 4 and 5 received 1 ml of 0.12 M sodium deoxycholate. Instillations were carried out three times per week for 18 weeks (Figure 10d:1).

In addition, rats in group 5 received metronidazole (22.5 mg/kg/rat/day; May & Baker Ltd., Dagenham, Essex) dissolved in the drinking water from the start of the instillations until the end of the experiment. Group 1 rats (controls) received neither intrarectal instillations nor metronidazole, and those in group 2 received metronidazole alone (Figure 10d:1).

Autopsy specimens

Rats were killed when moribund or at the end of 28 weeks. The following segments were thoroughly flushed with cold saline to remove all content: duodenum, jejunioileum and colorectum. Each segment was weighed and measured, and the number, size and position of all tumours were recorded. The wet weights of the caecum, liver, kidneys and spleen were also recorded.

In addition, a 1 cm segment of colon was excised 5-6 cm from the anus, and 5 μ m haematoxylin and eosin sections were prepared. The mean crypt depth in these specimens was estimated by ocular micrometry.

Results

Mortality rate

Eleven rats (10%) died before sacrifice either from colonic perforation during instillation (2 rats), haemorrhage secondary to duodenal or colonic cancer (2), haematuria (1), pneumonia (2), or cancer of the external auditory canal (1). In 3 rats that died during the early part of the experiment, the cause of death could not be determined. The yields of surviving animals at the end of the experiment are given in Figure 10d:1).

Body weight

Rats in all groups gained weight steadily until week 25, after which weights remained constant until sacrifice 3 weeks later. Neither intrarectal deoxycholate nor oral metronidazole had any consistent effect on body weight.

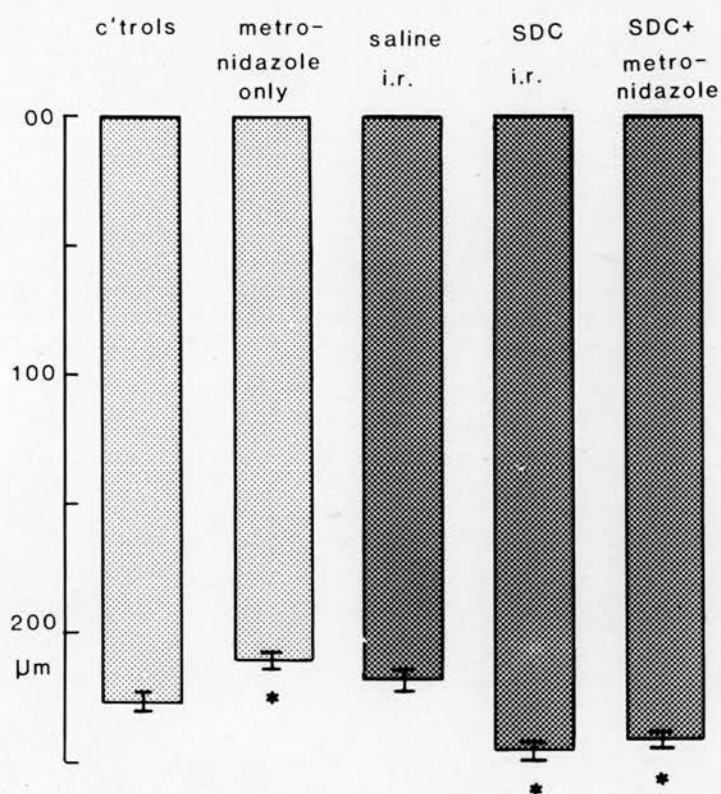
Intestinal adaptation

No differences between the groups were found in the lengths and weights of the duodenum, jejunum or ileum, nor in the weights of the caecum, liver, kidneys or spleen.

The mean colonic crypt depth in controls was $226 \pm 3 \mu\text{m}$ (mean \pm SEM) compared with $246 \pm 3 \mu\text{m}$ in the SDC irrigated group and $242 \pm 3 \mu\text{m}$ in the SDC + metronidazole group ($P < 0.001$; Figure 10d:3). Intrarectal saline had no effect on crypt depth compared with controls, but metronidazole alone produced a 9% decrease ($211 \pm 3 \mu\text{m}$; $P < 0.001$).

Intestinal tumours

All but 2-3 rats in each group developed one or more colorectal



crypt depth (mean ± sem) in descending colon

* $p < 0.001$ vs controls

FIG. 10d:3 Colonic crypt depths at sacrifice (28 weeks). SDC = sodium deoxycholate.

tumours (Figure 10d:4). Intrarectal deoxycholate almost trebled colorectal tumour yields from 2.4 ± 4 per rat (mean \pm SEM) in controls to 6.4 ± 0.5 ($P < 0.001$). Metronidazole reduced this effect by 33% ($P < 0.001$) but the tumour yield (4.2 ± 0.5) remained 75% higher than that in controls ($P < 0.001$). Neither metronidazole alone (2.2 ± 0.6 tumours per rat) nor intrarectal saline (2.8 ± 0.5) had any effect on colorectal carcinogenesis. No significant differences in tumour size were found between groups.

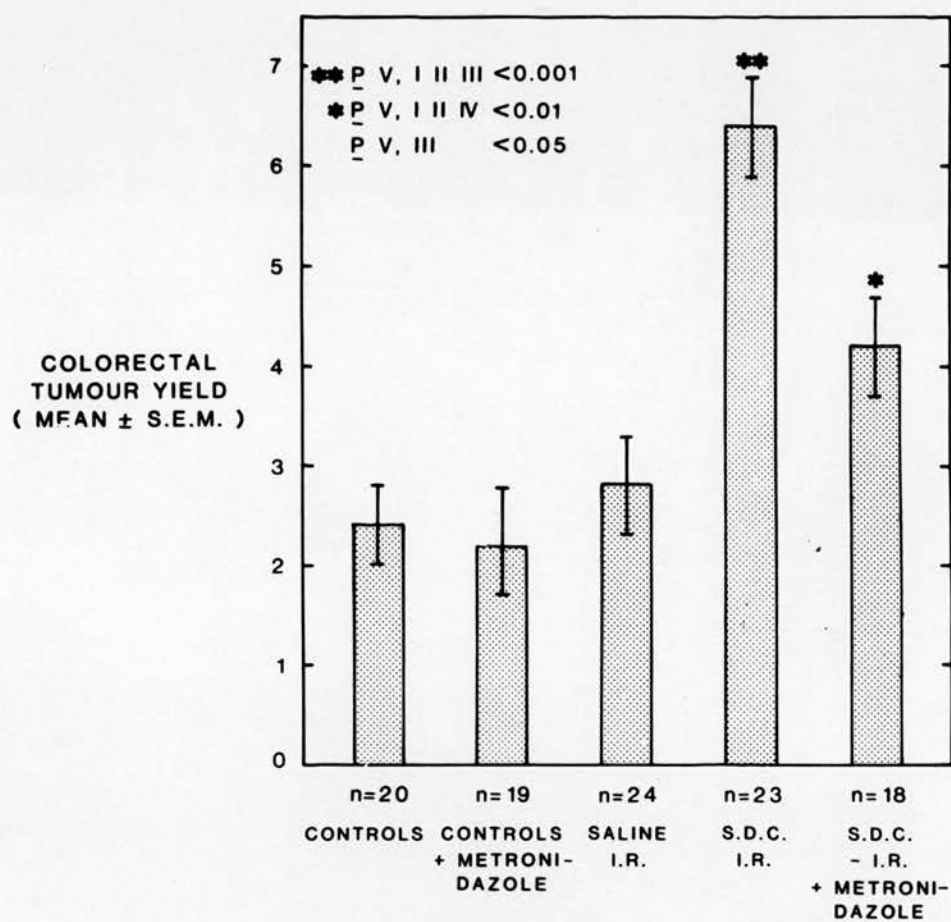
The overall pattern of colorectal tumour distribution was similar in the five groups. Ninety-six per cent of all tumours developed in the distal 60% of the large bowel (Figure 10d:5). The effect of SDC instillation was maximal in the distal 40% segment, where it produced a 193% increase in tumour yield over controls ($P < 0.001$), while the increase proximal to this was only 14%. Clearly the instillations were effectively reaching this distal 40% segment.

Tumours also arose in the duodenum ($n=5$), jejunum (3), and caecum (1), but their incidence was unaffected by SDC or metronidazole administration. In addition, 3 rats developed tumours of the external auditory canal, and metastases were found in the lung, liver and omentum.

Comment

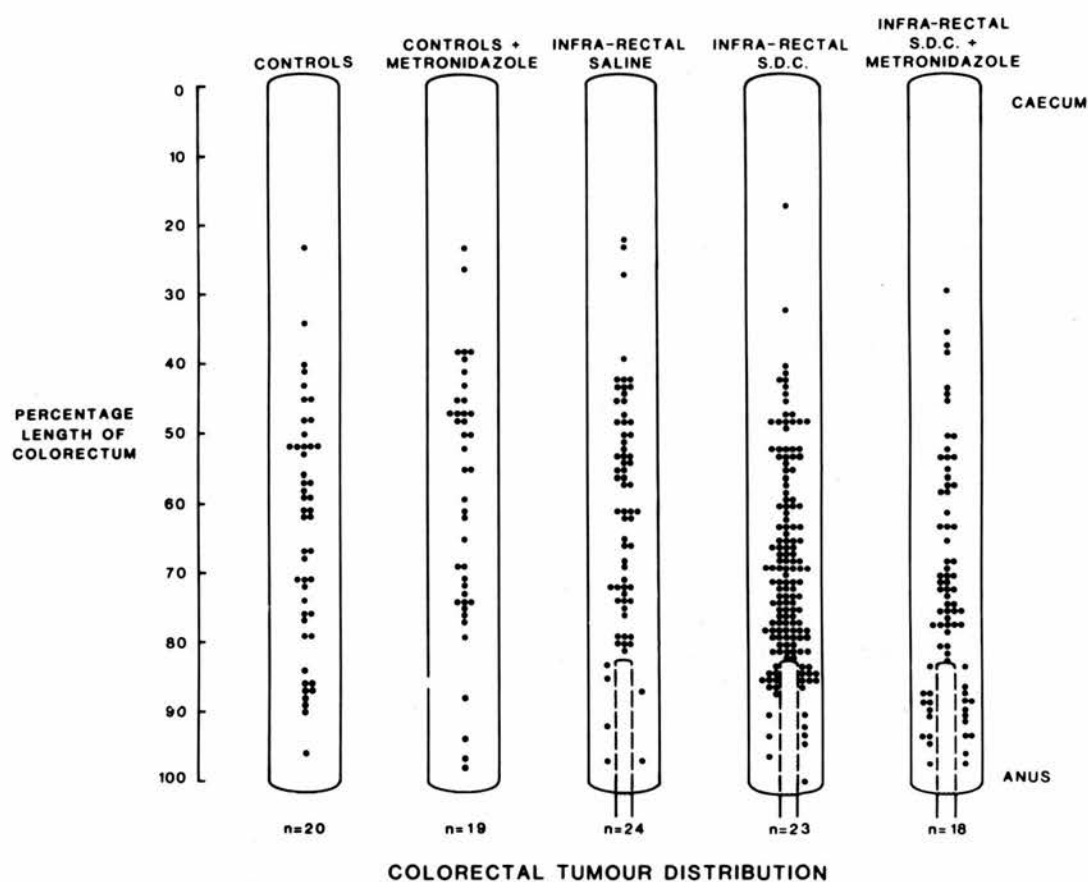
The data support the contention that sodium deoxycholate is a potent promoter of experimental colorectal carcinogenesis when instilled directly into the lumen of functioning large bowel (Narisawa et al, 1974; Reddy et al, 1977b). Bile acids are tropic to ileal mucosa (Williamson et al, 1978c), and the finding that SDC increases colonic crypt depth indicates that they produce a similar response in colonic mucosa. Hyperplasia is a strong promoter of experimental intestinal cancer (Williamson, 1982a; Barthold,

FIG.10d:4



COLORECTAL TUMOUR YIELD (MEAN \pm S.E.M.)
SDC=SODIUM DEOXYCHOLATE

FIG.10d:5



1981) (Chapter 7). The tropic effects of SDC on the mucosa might therefore be sufficient to explain its tumour-promoting effect.

In Experiment B, isolating a long segment of colon from the faecal stream as a Thiry-Vella fistula produced both mucosal hypoplasia and reduced susceptibility to azoxymethane. SDC instillation into this defunctioned colon had no effect on the reduced tumour yield or the mucosal hypoplasia. Clearly SDC requires the presence of faeces or some faecal constituent in order to exert its cocarcinogenic effects. The mechanical stimulus of faecal bulk may be important in maintaining normal mucosal cell turnover (Williamson, 1982b). Similarly a normal bacterial flora is necessary for maintenance of the normal mucosal proliferative state (Abrams et al, 1962) and its composition may modulate carcinogenesis (Hill, 1979). The bacterial population in a defunctioned Thiry-Vella fistula is probably very different both qualitatively and quantitatively from that in normal functioning colon.

In this study, metronidazole had no effect on carcinogenesis in response to azoxymethane alone. Yet Goldin and Gorbach (1981) have found that the administration of tetracycline or erythromycin to rats receiving dimethylhydrazine markedly reduces colorectal carcinogenesis; these antibiotics have a different spectrum of antibacterial activity than metronidazole. Since chemical carcinogenesis is also reduced in germ-free rats (Reddy et al, 1975b), it is possible that the dose of metronidazole did not reduce the population and metabolic activity of colonic bacteria enough to inhibit carcinogenesis. Nevertheless there was a slight but significant reduction in colonic crypt depth, similar to that found in the ileum of germ-free mice (Abrams et al, 1962).

The importance of faecal anaerobes in the cocarcinogenic role of bile

acids is highlighted by the finding that metronidazole partly suppresses the effect of intrarectal SDC. However, although metronidazole reduced the cocarcinogenic potential of intrarectal SDC, deoxycholate remained strongly cocarcinogenic. Since bile acids are also cocarcinogenic in germ-free rats, the presence of bacteria is clearly not essential (Reddy et al, 1977b).

EXPERIMENT E

Effect of bile acids on colonic mucosal cell proliferation and faecal bacterial population.

Background

In experiment D the instillation of sodium deoxycholate (SDC) into the functioning large bowel of the rat clearly promoted colorectal carcinogenesis induced by azoxymethane. The mechanism of this cocarcinogenic activity, which was not seen in defunctioned colon (Experiment B), has not been identified. The finding that SDC also increased mean colonic crypt depth suggests that its tumour-promoting potential might be linked to a tropic effect on the mucosa. This possibility is discussed in the preceding section and prompts further study.

Oral metronidazole reduces but does not abolish the cocarcinogenic effect of intrarectal SDC, supporting the view that faecal bacteria, particularly anaerobes, may be key intermediaries governing the cocarcinogenic role of bile acids (Aries et al, 1969; Hill, 1979). Metronidazole did not reverse the SDC-induced increase in crypt depth, but given alone it produced a decrease compared to controls. It seems likely, therefore, that metronidazole partly inhibits the promotional effect of SDC by a direct effect on colonic bacteria rather than by depressing SDC-induced mucosal hyperplasia.

Experiments designed to test the cocarcinogenic potential of bile acids by intrarectal instillation of these substances (Narisawa et al, 1974; Reddy et al, 1977b) (Experiment D) might be criticised on the grounds that the colorectal mucosa is exposed to doses and concentrations that are probably much greater than those ever found in the rat or human large bowel,

irrespective of dietary fat and cholesterol content. Moreover, the introduction of such strong chemical solutions into the colorectum could have major effects on the colonic microflora. If the composition of the colonic bacterial flora was altered or the population appreciably reduced, then conclusions drawn from these experiments about the role of bacteria in bile acid cocarcinogenesis would be of questionable validity.

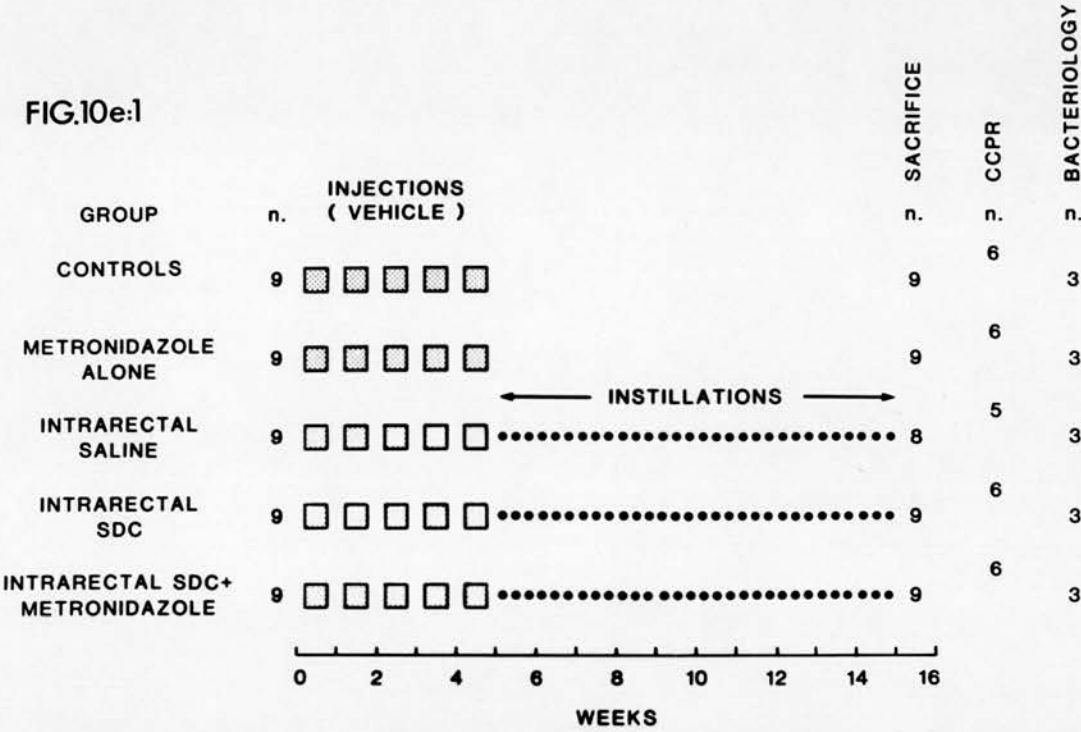
This experiment was designed with two main objectives. Firstly, the effects of intrarectal deoxycholate and oral metronidazole on the mucosal proliferative state were tested using the stathmokinetic technique for estimation of the crypt cell production rate (CCPR) in various intestinal segments. Secondly, total colonic bacterial counts were carried out to determine the effect of metronidazole alone and in combination with intrarectal SDC on the colonic bacterial population.

Design

Forty-five rats weighing 180-200 g, were allocated to five equal groups (1-5) (Figure 10e:1). All animals received a weekly subcutaneous injection of vehicle (sterile H₂O) for 6 weeks. None received carcinogen. Beginning one week later, intrarectal instillations were carried out thrice weekly as described in Experiment D in groups 3-5. Group 3 received 1 ml N saline, and groups 4 and 5 received 1 ml 0.12 M SDC. Group 5 also received metronidazole 22.5 mg/kg dissolved in the drinking water. Group 1 served as controls and group 2 received metronidazole alone. Instillations and metronidazole were continued for 10 weeks until week 17 when all animals were killed. Animals were weighed weekly throughout the experiment.

At sacrifice, 6 animals in each group received vincristine 1 mg/kg i.p. and were killed at half-hourly intervals from 30-180 minutes later. The

FIG.10e:1



EXPERIMENTAL DESIGN. NUMBERS IN EACH GROUP AT THE START OF THE EXPERIMENT AND SURVIVING UNTIL SACRIFICE ARE SHOWN

weights of the liver, kidneys and spleen were noted. The jejunoileal and colorectal segments were flushed clean with saline, their weights and lengths and the caecal weight were recorded before processing (as previously described) for estimation of the crypt cell production rate. CCPRs were subsequently determined in the following segments: terminal ileum, caecum, right middle and left colon and rectum.

In a further 3 rats from each group, the entire large bowel (including the caecum) and its contents were removed intact, placed in a sterile container and sent for bacteriological examination.

Bacteriological methods

Since facilities for full bacteriological investigation of the faecal flora were not available, specimens were processed by methods routinely available in a clinical laboratory.

Immediately on arrival in the laboratory, the faeces contained in the rat large bowel specimens were diluted 10-fold in heart infusion broth. Further 10-fold dilutions were made to 10^{-12} . The predominant bacteria were counted by the Miles and Misra technique (1938) on the following media and conditions of incubation: 5% horse-blood agar incubated in air with 5% CO_2 for 24 hours; MacConkey's bile salt-lactose agar in air for 24 hours; horse-blood agar with cysteine and neomycin anaerobically with 10% CO_2 in a conventional anaerobic jar for 48 hours.

In the Miles and Misra technique, dropping pipettes delivering 50 drops/ml were used. Starting with the highest dilution plates containing the above media received a drop (0.2 ml) of each dilution onto a marked segment from a fixed height. The drop areas from the higher concentrations yielded circular patches of confluent growth. Counts were made in areas

containing the largest number of colonies without signs of confluence or of gross diminution of colony size due to overcrowding.

In addition to this technique, spread plates were also prepared to assist in recognition of bacteria present. Each colonial form was counted separately, Gram-filmed and identified to general categories.

Results

Mortality

One rat in group 3 (i.r. saline) died from colonic perforation in week 12.

Body weight

Rats in all groups gained weight steadily throughout the experiment. No significant differences in body weight were found between the groups, although animals receiving i.r. SDC (groups 4 and 5) weighed slightly less at sacrifice than those in the other three groups (Table 10e:1).

Intestinal adaptation

No differences between the groups were found in the lengths and weights of the jejunoileal and colorectal segments or in the weights of the caecum, liver, kidneys and spleen (Table 10e:1).

There were minor fluctuations between the groups in CCPR in the terminal ileum (Figure 10.e:2a). Metronidazole given alone decreased the CCPR in the caecum by 51% ($\underline{P} < 0.01$ vs controls), produced smaller 40-47% reductions in the colonic segments and had no effect on rectal CCPR (Figure 10.e:2a-f). Intrarectal saline had no effect on the ileum, caecum or colon but there was a 70% increase in rectal CCPR ($0.5 < \underline{P} < 0.1$).

Intrarectal deoxycholate with and without concurrent metronidazole increased CCPRs in the colonic segments and the rectum. Increments were greatest in the rectum (221 and 364%; SDC and SDC plus metronidazole, respectively) and least in the right colon (57 and 96%) (Figure 10.e:2c-f). There were no consistent differences in CCPR between the SDC alone group and the SDC plus metronidazole group in any of the segments examined.

	1	Controls	2	Metronidazole Alone	3	I.R. Saline	4	I.R. SDC	5	I.R. SDC + Metronidazole
<u>No</u>		9		9		8		9		9
<u>Body Weight</u>		545.6 ± 47.0		573.2 ± 60.1		556.8 ± 68.7		521.9 ± 77.1		514.3 ± 58.8
<u>Jejunioileum</u>										
Length		132.0 ± 3.3		129.3 ± 6.2		134.6 ± 9.6		128.0 ± 2.5		130.2 ± 3.8
Weight		10.9 ± 0.7		10.3 ± 1.0		10.7 ± 1.9		9.6 ± 1.1		12.6 ± 3.1
<u>Caecum</u>										
Weight		1.5 ± 0.1		1.5 ± 0.1		1.6 ± 0.5		1.4 ± 0.1		1.6 ± 0.2
<u>Colorectum</u>										
Length		29.7 ± 1.0		29.5 ± 1.4		29.5 ± 1.9		28.8 ± 1.2		28.7 ± 1.0
Weight		2.8 ± 0.2		2.6 ± 0.3		2.5 ± 0.2		2.7 ± 0.2		2.7 ± 0.5
<u>Liver</u>										
		15.6 ± 1.2		15.8 ± 2.5		18.2 ± 2.3		14.8 ± 1.0		16.4 ± 2.0
<u>Kidneys</u>										
		3.5 ± 0.5		3.4 ± 0.4		3.7 ± 0.5		3.5 ± 0.4		3.4 ± 0.4
<u>Spleen</u>										
		1.2 ± 0.2		1.0 ± 0.1		1.0 ± 0.1		1.0 ± 0.1		1.0 ± 0.1

Table10e:1 Body weight (g), lengths (cm) and weights (g) of small and large bowel, and weights caecum, liver, kidneys and spleen in groups 1-5 at sacrifice (mean ± S.D.).

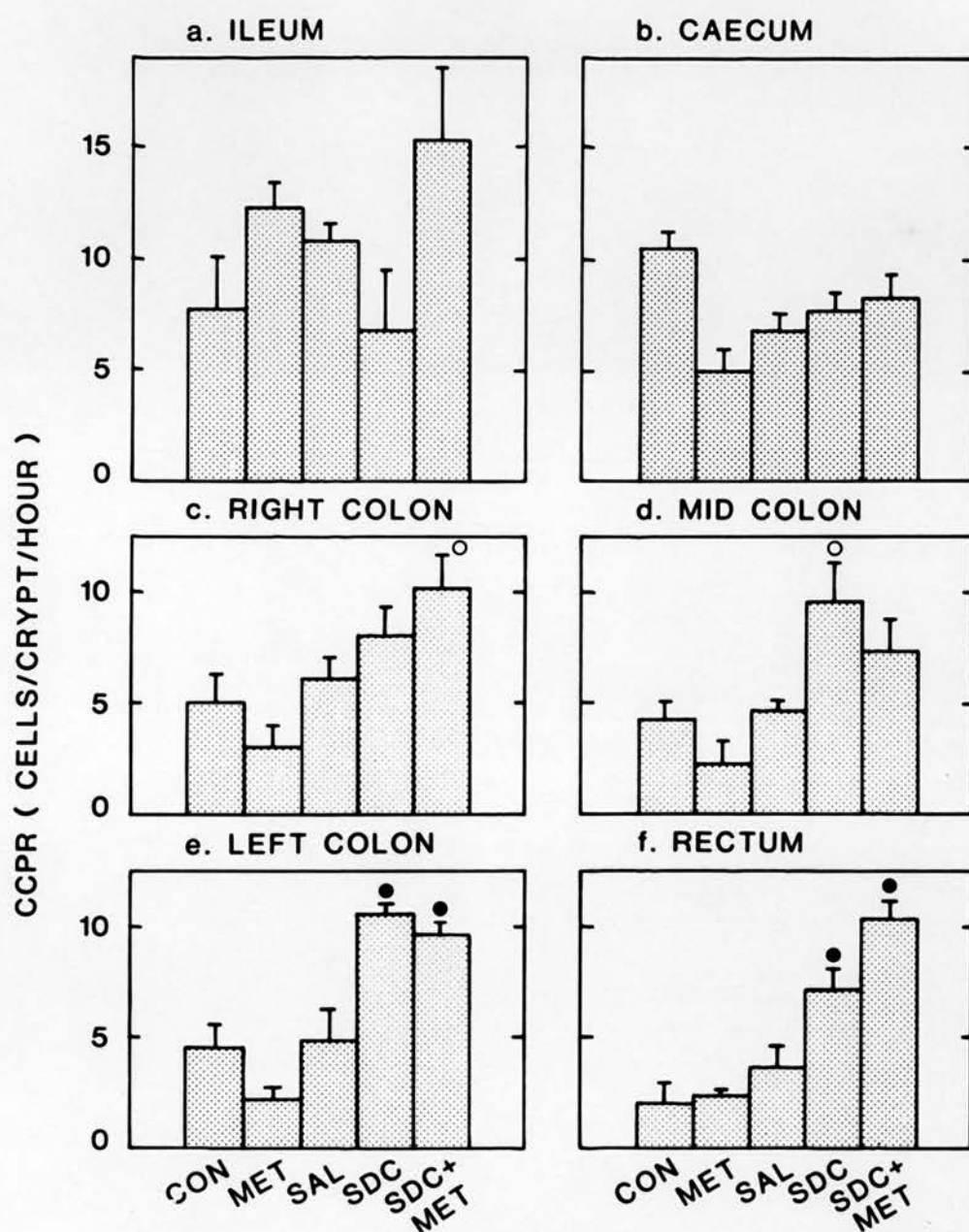


FIG.10e:2 a-f CCPR (+S.E.) IN 6 INTESTINAL SEGMENTS IN THE 5 GROUPS

CON=CONTROLS, MET=METRONIDAZOLE ALONE,
SAL=IR. SALINE, SDC=IR. SDC

○ $P < 0.05$ vs CONTROLS ● $P < 0.01$ vs CONTROLS

Neither the caecum, nor the ileum were significantly affected by SDC with or without metronidazole.

Colonic Bacterial Counts

No consistent differences were found between the groups in the numbers or types of bacteria identified (Figure 10.e:3). Although no clostridia were detected in groups 2-5, these were also absent in one of three control rats.

Comment

This study confirms the impression given by the crypt depth data in Experiment D that intrarectal SDC produces hyperplasia of the large bowel mucosa independent of the stimulatory effect induced by azoxymethane in Experiment A. It is interesting that hyperplasia was detected as far proximal as the right colon, but not in the caecum or the ileum. The detergent property of the SDC solution probably enabled some of the instillate to reach the proximal colon, although the maximal tropic effect was observed in the left colon and rectum where the cocarcinogenic effect was most clearly evident in Experiment D.

The mechanism by which SDC induces hyperplasia is not clear but is paralleled by the tropic effect of bile observed in the ileum following pancreaticobiliary diversion to mid-small bowel (Williamson et al, 1978c). It is unlikely that bile is directly trophic or nutritive to colorectal mucosa but it may produce irritation and inflammation leading to hyperplasia similar to that seen adjacent to anastomotic suture lines (Rijke et al, 1977; Williamson, 1978a). Hyperplasia does predispose to neoplasia (Barthold, 1981; Williamson, 1982a) (Experiment A) and, whatever its cause, the

FIG.10e:3 a-e

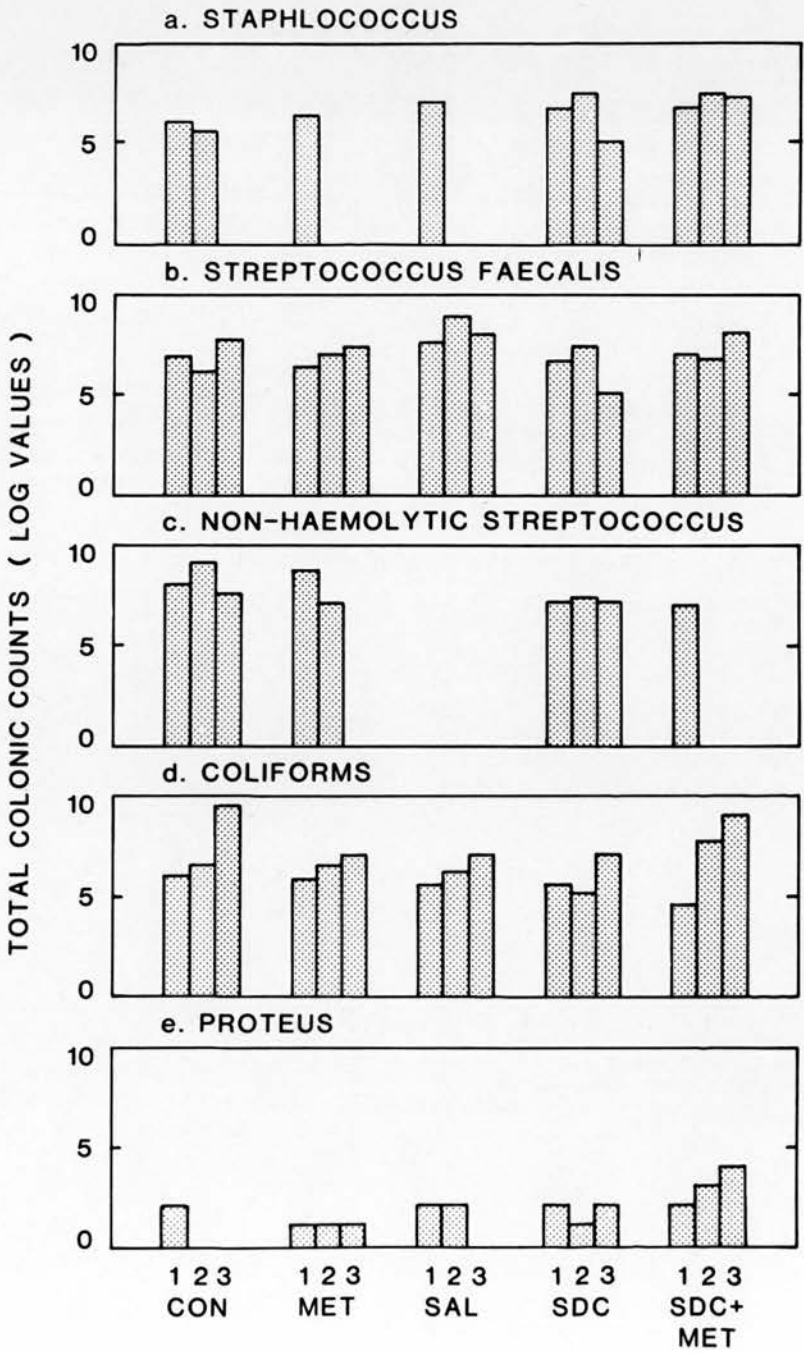
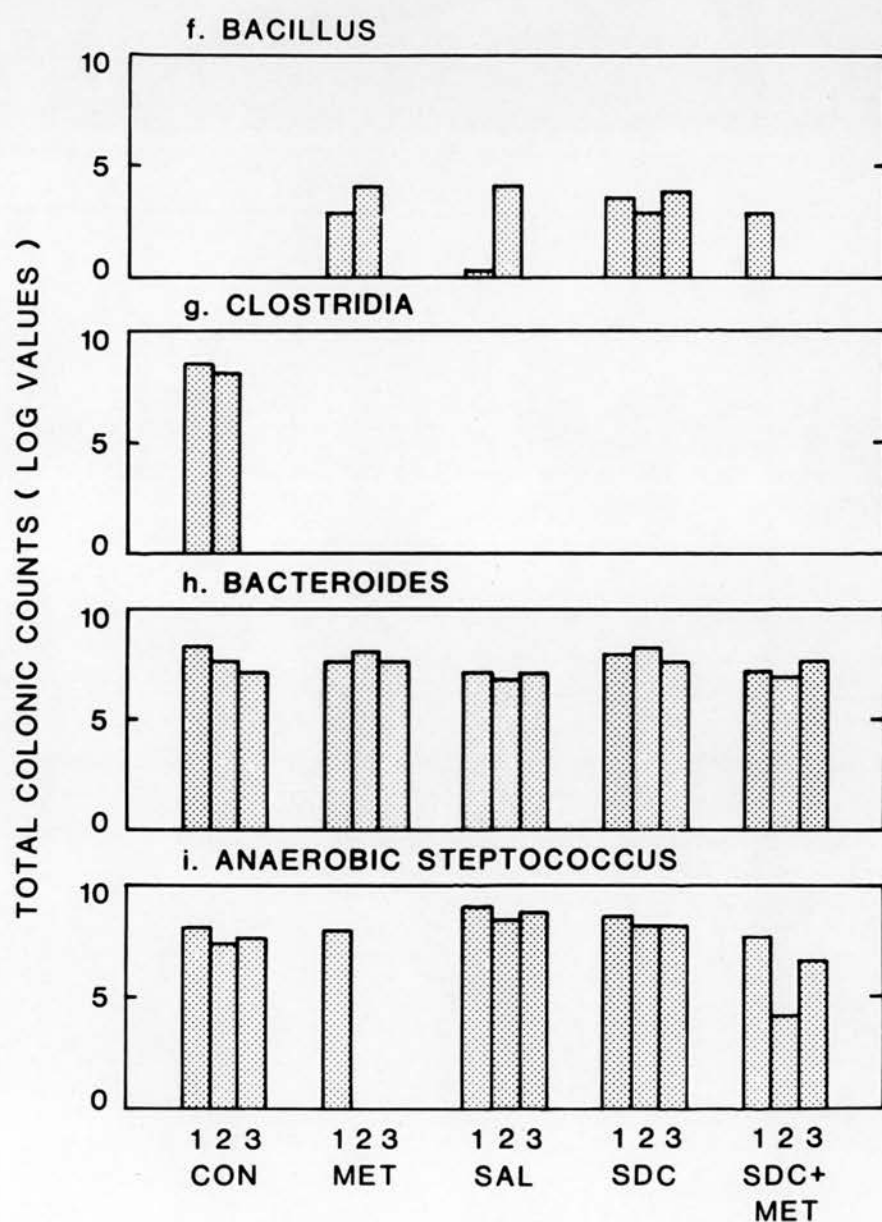


FIG.10e:3 f-i



a-i TOTAL COLONIC BACTERIAL COUNTS
IN 3 RATS FROM EACH GROUP (LOG VALUES)

colonic mucosal hyperplasia seen in this model may explain the cocarcinogenic effect of SDC. Inflammation may also be an important promoting factor in neoplasia. In man the inflammation of ulcerative colitis greatly enhances the risk of colorectal cancer (Lennard-Jones et al, 1977; van Heerden and Beart, 1980).

The results of this experiment contrast with those of Experiment B, where SDC in the same concentration had no effect on the mucosal atrophy found in defunctioned colon. The mechanical stimulus of faecal bulk or the presence of other factors in the faeces may be necessary for bile to exert its tropic effect and hence its tumour-promotion.

Oral metronidazole does not reverse the tropic effect of SDC and therefore its partial inhibition of SDC tumour-promotion is probably due to a direct action on bacteria capable of degrading bile acids to more active metabolites. The reduction in caecal and colonic CCPRs produced by metronidazole given alone may be due to a disturbance of the normal bacterial population, the "physiological flora" which is necessary for the maintenance of normal mucosal cell proliferation (Abrams et al, 1962).

The limited bacteriological results of this experiment do not clearly support or refute any hypotheses regarding the role of bacteria and the action of metronidazole in this model. There are several reasons for this. Financial and staffing restraints allowed only small numbers of animals to be used and dictated the use of techniques that probably precluded detection of all but the most robust anaerobes. Isolation of more sensitive anaerobes would have involved the use of evacuated tubes and incubation in nitrogen on expensive selective media. Moreover, bacteria in the colonic mucosa itself may be quite different in type and metabolic activity to those in the faecal stream and they may have escaped detection among the large

numbers of bacteria cultured from the faecal stream. Metronidazole may reduce the metabolic activity of anaerobes without a measurable reduction in their numbers.

Nevertheless, within these limits it is clear that SDC instilled intrarectally in rats regularly over a period of months does not affect faecal flora so profoundly as to call into question the relevance of the model to the role of bile acids in human colorectal cancer.

The 18 rats in this study receiving i.r. SDC and vehicle served as a further control group for Experiment D. No tumours developed in these rats, which suggests that while bile acids are strong promoters of colorectal carcinogens they do not initiate neoplasia.

EXPERIMENT F

Caecal carcinogenesis following distal transposition.

Background

In Experiments A, B and D the colon and rectum have proved sensitive to initiation of carcinogenesis by azoxymethane and to the cancer-promoting effects of manoeuvres which stimulate mucosal hyperplasia. The caecum by contrast, seems relatively resistant to azoxymethane initiation, and the moderate hyperplasia induced by ileal resection and bypass, or colonic bypass, does not appear to promote carcinogenesis. Indeed, out of 365 rats which received azoxymethane in these experiments only 6 (1.6%) developed caecal tumours.

The rat caecum shares its innate resistance to carcinogenesis with its human counterpart, the appendix. Several other experimental studies reveal a much lower incidence of tumours at this site than in the rest of the large intestine (Williamson et al, 1979; Bristol et al, 1984; Scudamore and Freeman, 1983). In some reports the caecum has been completely spared, despite appreciable numbers of tumours arising elsewhere in the intestinal tract, particularly the distal colon (Williamson et al, 1978d, 1980a,b). Surgical shortening of the gut by resection or bypass is a powerful stimulus both to adaptive hyperplasia and carcinogenesis in the residual functioning bowel; but while it appears to promote caecal hyperplasia, its effect on caecal carcinogenesis is minimal (Williamson et al, 1980a; Williamson, 1982a).

The luminal environment of the caecal mucosa might be responsible for maintaining this resistance to neoplasia. The composition, bulk, transit time and bacterial population of the faeces passing through the caecum

could each be important in this respect. The present study was designed to test the effect of exposure to the distal faecal stream on the proliferative state of the caecal mucosa and its susceptibility to chemically-induced carcinogenesis. Since operation could affect body weight and intestinal blood flow, caecal transposition was carried out after the azoxymethane injections to avoid differences in the dose of carcinogen and its bloodborne delivery to the adapting bowel.

Design

Experimental animals

Fifty rats weighing 100-140 g were randomised to 2 groups. All animals received weekly subcutaneous injections of azoxymethane 15 mg/kg for 6 weeks. Each rat was submitted to operation 7-12 days after the last injection of carcinogen.

Caecal transposition (n=30) was performed as follows. The ascending colon and terminal ileum were transected 0.5 cm from their junctions with the caecum, and the ileal stump attached to the caecum was ligated with a 3/0 silk suture (Figure 10f:1b). The distal colon was transected at the pelvic brim, and the caecum was mobilised on its blood supply and inserted in an isoperistaltic direction between the cut ends. The stump of the ascending colon was anastomosed to the rectum. A large part of the caecum is normally a blind loop, so to expose its entire mucosa evenly to distal colonic content, the descending colon was anastomosed to an apical caecotomy rather than to the ileal stump. An ileocolic anastomosis restored intestinal continuity.

Sham transposition (n=20) involved transection and reanastomosis at equivalent points of the proximal and distal colon, with caecotomy and

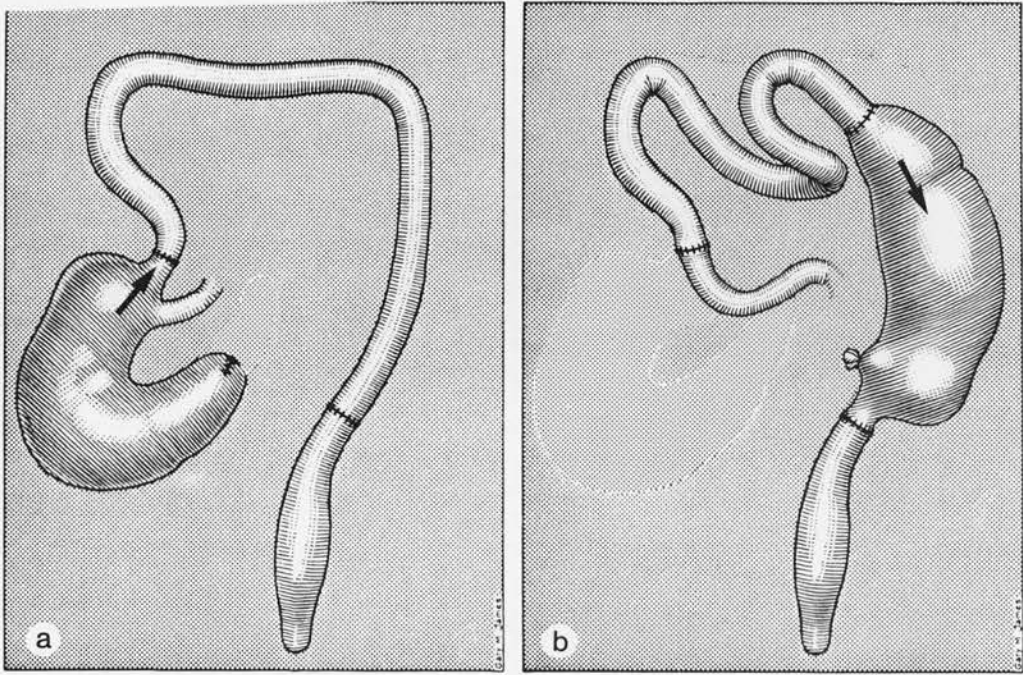


FIG 10f:1 Operations: A = sham transposition B = caecal transposition



FIG 10f:2 Caecal tumour at site of resutured caecotomy.

resuture at the caecal apex (Figure 10f:1a).

Autopsy specimens

Rats were regularly examined for evidence of tumour development and were killed when moribund or at the end of 28 weeks. The following segments were thoroughly flushed with cold saline to remove all content: duodenum, jejunioileum, caecum, colon (proximal to transposed caecum or between anastomoses) and rectum. The number, size and position of all tumours were recorded. The surface area of the caecum was estimated as previously described (Chapter 9) and the length and weight of the other intestinal segments was determined. The weights of the liver, kidneys and spleen were also recorded.

In addition, 6 rats in each group given vincristine (1 mg/kg i.p.) were sacrificed at 30-minute intervals thereafter up to 180 minutes. Specimens from these animals were processed for estimation of the crypt cell production rate (Chapter 9). The CCPR was determined in the following segments: ileum, caecal apex, distal caecum, proximal colon, distal colon and rectum.

Results

Mortality rate

There were 3 early deaths (6%) from anaesthetic overdose. Another 6 rats (12%) died before sacrifice, 3 from adhesion obstruction and 3 from intestinal cancer. Premature mortality rates were 20% after caecal transposition (24 survivors) and 15% after sham transposition (17 survivors).

Body weight

Following sham transposition, rats lost 3% of body weight during the first 48 hours but regained their original weight by the end of the first postoperative week. Following caecal transposition, rats continued to lose weight (7%) during the first week and only regained their preoperative weight at the end of the second week. Although they consistently weighed a little less than shams throughout the remainder of the experiment, their weight was not significantly different at sacrifice (557 ± 12 g vs 587 ± 6 g; mean \pm SEM).

Intestinal adaptation

The surface area of the transposed caecum was 21% greater than that of the orthotopic caecum. In the colorectum both total weight and total length were greater after transposition than after sham transposition. No differences were found in the length and wet weight of the duodenum and jejunioileum nor in the weights of the liver, kidneys and spleen. Crypt cell production rates (CCPR) in the colon were similar in each group (Table 10f:1). Transposition increased CCPR in the terminal ileum by 31% and reduced it in the rectum by 62%. No consistent effect was observed in the caecum.

Intestinal tumours

There were no tumours in the transposed caecum, but 3 developed in the orthotopic caecum at the site of caecotomy closure (Figure 10f:1). Suture-line tumours were common elsewhere, particularly at the colocaecal and caecorectal anastomoses after transposition and at the distal colonic anastomosis in shams (Figure 10f:3). The overall yield of suture-line cancers after transposition (1.8 ± 0.2 per rat; mean \pm SEM) was twice that in shams

	Controls	Transposition
Ileum	10.6 \pm 0.4	13.9 \pm 1.0*
Caecal apex	6.9 \pm 2.2	9.5 \pm 1.2
Distal caecum	13.9 \pm 0.5	5.7 \pm 3.3
Proximal colon	12.5 \pm 3.1	10.0 \pm 1.9
Distal colon	9.4 \pm 0.8	11.5 \pm 4.5
Rectum	13.8 \pm 2.0	5.2 \pm 1.5**

Table 10f:1

Crypt cell production rates (cells/crypt/hour) after sham caecal transposition (controls) and after caecal transposition (means \pm SEM).

* $\underline{P} < 0.02$ ** $\underline{P} < 0.01$.

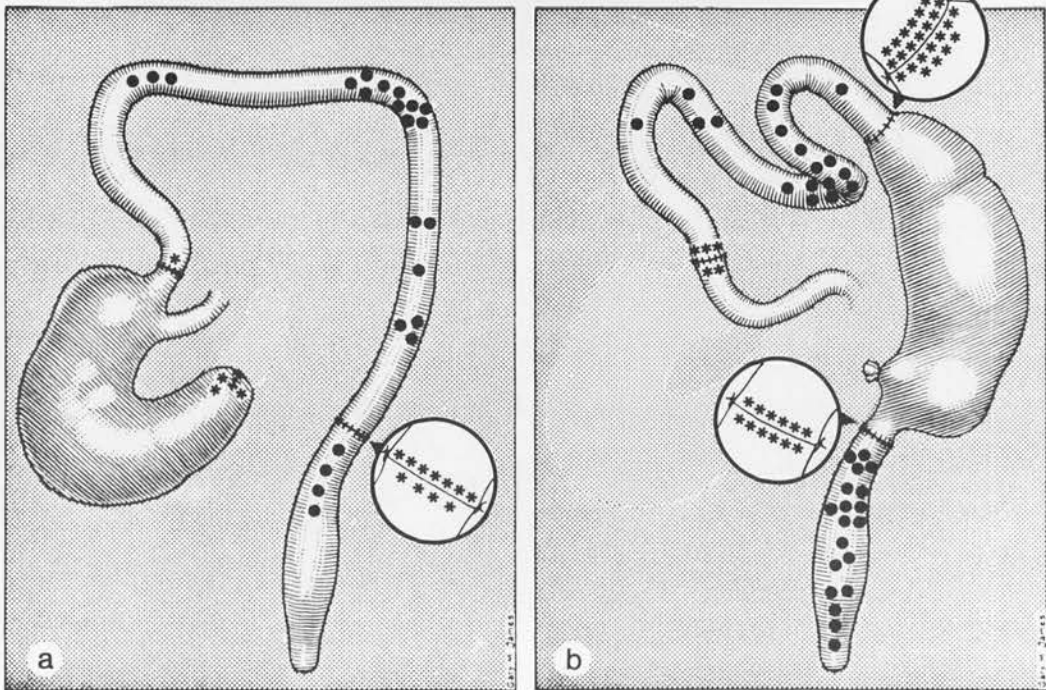
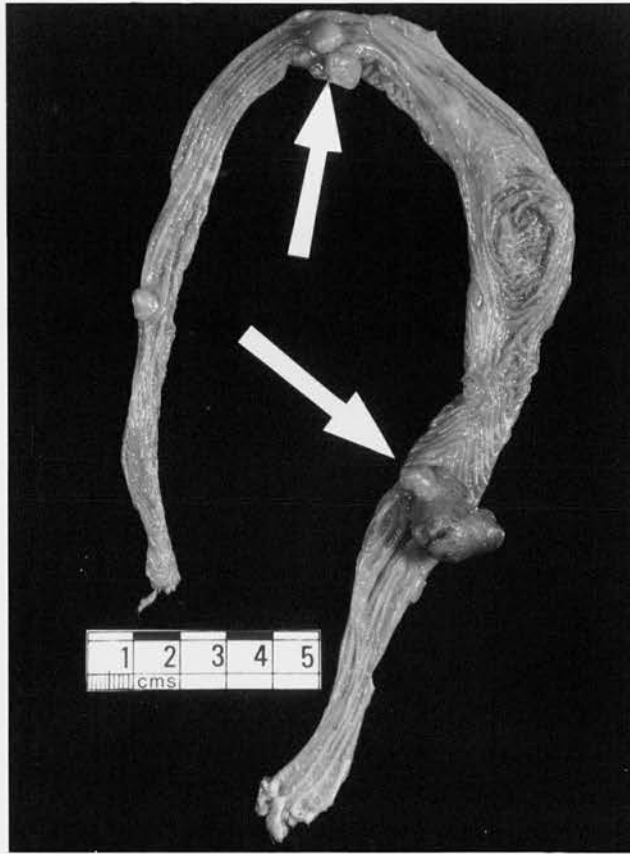


FIG. 10f:3 Tumours at colorectal and caecorectal anastomoses after caecal transposition. Overall distribution of tumours after sham transposition is shown in a and that after caecal transposition in b.

(0.9 ± 0.3 ; $P < 0.05$). The yield of non-anastomotic colorectal tumours in the shams (1.4 ± 0.3) matched that after transposition (1.5 ± 0.2), but their distribution was different (Figure 10f:3). Tumour yield in the rectum was increased 4-fold from 0.2 ± 0.1 in shams to 0.8 ± 0.2 in the transposition group ($P < 0.05$).

Irrespective of operation tumours were also found in the duodenum (7 rats), upper jejunum (2), external auditory canal (1) and at metastatic sites (13).

Comment

Rat caecum maintains its innate resistance to azoxymethane-induced carcinogenesis when exposed to the luminal environment of the distal colon, where tumours most commonly arise. Any differences between these two regions in respect of faecal bulk or contents, such as bile acids and bacteria, would surely have been lessened if not abolished by caecal transposition. In determining susceptibility to neoplasia, therefore, luminal influences seem less important than the inherent characteristics of the mucosa, assuming that they exert their effect at the stage of promotion rather than initiation. This assumption is supported by Experiment B and by previous studies exploring the role of intestinal adaptation in experimental carcinogenesis (Williamson et al, 1978d, 1982).

The caecum shares its relative resistance to neoplasia with the distal small bowel (Williamson, 1982a). Segments of mid-jejunum transposed to the colon do not develop cancer, even though several tumours can be induced in the adjacent large bowel (Gennaro et al, 1973; Celik et al, 1981). Conversely, colonic segments transposed to the small bowel retain their normal susceptibility to carcinogenesis.

Adaptive hyperplasia is no more able to overcome the resistance of insusceptible bowel to experimental carcinogenesis than altered luminal contents. The ileum becomes intensely hyperplastic after jejunal resection, pancreatobiliary diversion or subtotal colectomy, but virtually no ileal tumours develop whether operation precedes or follows the administration of azoxymethane (Williamson et al, 1978c, 1980b). Resistance to carcinogenesis may be attributable to local epithelial defence mechanisms or to the absence of enzymes needed to synthesise the ultimate carcinogen (Gennaro et al, 1973; Williamson, 1982a). Similar protective mechanisms could operate in the rat caecum. Although transposition produces relatively minor fluctuations in caecal CCPR, ileal resection stimulates caecal growth without altering tumour yields (Williamson et al, 1980a).

The finding of 3 suture-line tumours at the apex of the caecum in controls is interesting. In a similar experiment Pozharisski (1975) found that the presence of a nonabsorbable suture placed through the caecal wall enhanced caecal carcinogenesis, though to a much greater extent. Thus, local inflammation and hyperplasia seem more important in carcinogenesis than faecal composition. Suture-line tumours are a common feature both in patients surviving partial colectomy (Cohn, 1967) and in rats given chemical carcinogens either pre- or post-operatively (Williamson et al, 1979, 1982; Williamson, 1982a). Anastomotic recurrence in man could reflect chronic inflammation and hyperplasia caused by the suture material, inadequate resection, or implantation of viable cancer cells at operation (Cohn, 1967; Rosenberg, 1979; Williamson, 1982a; Umpleby et al, 1984). In both man and the rat anastomoses involving distal colon are at greater risk than those involving proximal colon or ileum (Wright and Cleveland, 1969; Williamson et al, 1982), presumably because of the left-sided predominance of large-

bowel cancer in each species. In the present study anastomotic tumours were more frequent after caecal transposition, in which two anastomoses contained a distal colonic component, than after sham transposition, when only one anastomosis had such a component.

Caecal transposition produced a slight increase in the length and wet weight of the colon and in the surface area of the caecum, without corresponding increases in cell proliferation. Perhaps an alteration in faecal bulk produced dilatation of the heterotopic caecum and consequent back pressure, leading to muscular hypertrophy. The increased tumour yield found in the rectum distal to the transposed caecum, despite paradoxical hypoplasia, is puzzling. Insertion of a large reservoir just proximal to this segment may have altered faecal composition in such a way as to facilitate tumour growth while partly usurping rectal function and thus depressing its mucosal cell turnover.

CHAPTER 11
GENERAL DISCUSSION

PART I

Intestinal Adaptation

Several aspects of intestinal adaptation to loss of functioning bowel have been explored in this work. These experiments confirm that loss of functioning bowel produces adaptive hyperplasia in bowel remaining in continuity (Experiments A,C) (Williamson, 1978, 1982a). The phenomenon of intestinal adaptation to various surgical operations has been much more extensively studied and documented in the small bowel than in the large bowel, but the evidence to date suggests that the colon is less responsive to adaptive stimuli (Bristol and Williamson, 1984). Although adaptation appears to affect the whole of the residual functioning bowel (including the stomach), it is maximal in bowel adjacent to the resected or bypassed segment (Experiments A,C) (Williamson et al, 1978a,d; Oscarson et al, 1979; Williamson, 1978,1982b). This pattern was confirmed in Experiments A and C. Both distal small-bowel resection or bypass (DSBR/B) and colonic bypass (TVF) produced mucosal hyperplasia which was maximal in segments immediately proximal and distal to the line of resection. In both cases, however, the caecum was initially slow to respond. Although immediately distal to the anastomosis after DSBR/B, caecal hyperplasia did not match that in the proximal colon until 30 weeks postoperatively. After TVF, CCPRs were increased in the ileum and rectum by 4 weeks but not in the caecum until 16 weeks. Adaptation in this segment appears more dilatory than in the small bowel, where changes can be detected within 48 hours of enteric resection (Obertop et al, 1977; Oscarson et al, 1977; Williamson et al, 1978a). The reason for this discrepancy is unclear; perhaps caecal mucosa requires a more sustained stimulus than the small bowel or colon before hyperplasia develops, which might partly explain its relative

resistance to carcinogenesis (Experiment F). Caecal mucosa appears to react independently of the muscle coat, the mass of which may therefore be influenced by different factors. While both DSBR/B and TVF increased caecal CCPR, only the former procedures increased its weight and surface area. On the other hand, these parameters were significantly increased by distal transposition, which had little effect on mucosal CCPR.

Adaptation to DSBR/B was sustained and even appeared to be increasing up to 30 weeks postoperatively in both jejunioileum and the large bowel. While lasting hyperplasia has been found consistently in the small bowel, the adaptive response of the large bowel has generally been regarded as a more transient phenomenon. Proximal, mid- and distal enterectomies all stimulate caecal and colonic hyperplasia 2-4 weeks postoperatively, but more prolonged adaptation has rarely been recorded (Tilson et al, 1976b; Nundy et al, 1977; Obertop et al, 1977; Williamson et al, 1978a; Tilson and Livstone, 1980; Bristol and Williamson, 1984)

Several explanations have been offered for the relatively temporary nature of colonic adaptation to enteric resection or bypass. The physiological need for colonic adaptation might be less than that for enteric adaptation, which would seem an appropriate response to reduced capacity for digesting and absorbing nutrients; functions not normally associated with the large bowel (Flint, 1912; Bristol and Williamson, 1984). Mucosal proliferation in the colon might be controlled by a different set of factors, luminal or systemic, to those that operate in the small bowel. For example, the mechanical stimulus of faecal bulk is more important in the maintenance of normal mucosal cell turnover in the large bowel than in the small gut (Dowling et al, 1967; Stragand and Hageman, 1977a,b) and raised levels of enteroglucagon which stimulates enteric hyperplasia (Sagar et al,

1983) have no effect on the proliferative state in isolated loops of colon (Bristol et al, 1982a). Alternatively it might be argued that once small bowel adaptation is established, there is no further need for the colon, which makes up only a minor proportion of the total intestinal surface area, to adapt. This could explain why proximal enterectomy, a powerful stimulus to ileal adaptation, has only a limited effect on the large bowel (Obertop et al, 1977; Tilson et al, 1976; Williamson et al, 1978a,d; Tilson and Livstone, 1980). Lasting effects have only been observed after distal enteric resection or bypass, procedures which leave no functioning small bowel distal to the line of resection capable of compensating for the missing segment (Experiment A) (Scarpello et al, 1978; Williamson et al, 1980a). Admittedly in two of these studies colonic adaptation may have been supplemented by the known hyperplastic stimulus of a chemical carcinogen (Experiment A) (Williamson et al, 1980a).

The response of the large bowel to colonic resection and bypass is even less impressive than its response to loss of functioning small bowel (Williamson et al, 1982). Colonic bypass produced moderate hyperplasia in the ileum and rectum by 4 weeks which had waned by 16 weeks when moderate caecal hyperplasia was observed (Experiment C). Ileal adaptation to partial or total colectomy is well established in rats (Buchholtz et al, 1976 ; Scarpello et al, 1978; Wright et al, 1982), and has also been observed in man (Wright et al, 1969a), but adaptation in residual large bowel is less striking. Left hemicolectomy may produce moderate increases in weight and mucosal DNA and protein content in the right colon at 40 weeks (Williamson et al, 1982). There is no convincing evidence that right hemicolectomy or caecectomy alone produce any appreciable response in the residual colorectum (Scarpello et al, 1978; Williamson et al, 1982). The

rectum appears to adapt to subtotal colectomy and ileorectal anastomosis, an operation which resembles TVF-colonic bypass in the extent of colon excluded from the faecal stream (Owen and Lyttle, 1979). Small bowel resection and bypass have similar late adaptive effects (Experiment A) (Williamson et al, 1978a), and the same may hold true for colonic bypass and resection.

As an organ the large bowel may have a greater functional reserve than the small bowel with less need to adapt to tissue loss. Furthermore, the hyperplastic ileum may take over part of the absorptive function of the resected or bypassed colon, thereby reducing the necessity for colonic adaptation.

Luminal Factors

In the small intestine luminal factors seem more important than systemic factors in the control of adaptation (Chapter 6). Yet bypassed distal ileum (DSBB) deprived of luminal contents did not display a statistically significant reduction in CCPR (Experiment A). This lack of demonstrable ileal atrophy has previously been observed after both enteric bypass and the creation of an ileal Thiry-Vella fistula (Williamson and Bauer, 1978; Williamson et al, 1980b). Food appears to maintain the normal aboral gradient of mucosal mass and brush border enzymes associated with nutrient absorption (Altmann and Enesco, 1967; Freeman et al, 1978c). The jejunum is normally exposed to chyme richer in nutrients than that in the ileum and is correspondingly much more sensitive to deprivation of normal content, whether by starvation (with or without parenteral nutrition) or by removal from normal intestinal continuity (Altmann, 1972; Levine et al, 1974; Williamson et al, 1978a). Alternatively, bacterial colonisation of the

bypassed ileum could reverse or prevent mucosal hypoplasia, though this is unlikely to have occurred after DSBB in which the ileocaecal valve was preserved.

The role of luminal factors in colonic adaptation is poorly defined. As digestion and absorption of nutrients are usually completed in intact small bowel, they may not have any direct tropic action on the large bowel. The late caecal response to DSBB/R and the moderate rectal hyperplasia following TVF could reflect tropic activity of luminal constituents rather less potent than nutrients (Experiments A,C). Whatever their influence in colonic hyperplasia, luminal contents play an important part in the maintenance of normal mucosal cell turnover. Colon defunctioned as a Thiry-Vella fistula shows marked atrophy, as evidenced by reduced wet weight and length, shorter crypts and moderately reduced crypt cell production rates (Experiments B,C). Similarly, prompt marked atrophy develops in defunctioned colon distal to a transverse colostomy (Tilson et al, 1976; Rijke et al, 1979) which is precisely reversed following its closure (Terpstra et al, 1981). Mucosal proliferation in the proximal colon seems more sensitive to extrinsic influences than that in more distal bowel. Right colonic adaptation is seen after left hemicolectomy but not vice-versa (Williamson et al, 1982), and in TVFs, CCPRs were reduced earlier in the proximal segment than in the more distal defunctioned colon (Experiment C).

The importance of faecal bulk on colonic cell proliferation is highlighted by the observation that irrigation of a defunctioned TVF with dilute faeces restores crypt depth to the level found in normal colon-in-continuity (Experiment B). Indeed bulk appears to be the most important factor maintaining normal mucosal cell turnover (Stragand and Hagemann,

1977a,b; Bristol and Williamson, 1984). The administration of a non-absorbable, non-nutritive bulk diet to rats preserves colonic mucosal integrity (Dowling et al, 1967). Conversely, low residue diets produce mucosal hypoplasia (Janne et al, 1977), even when caloric intake is identical to the standard diet (Heitman, 1983).

Other dietary constituents may have a part in the preservation of colonic mucosal mass. Mineral salts, particularly iron, are among substances implicated (Stragand and Hagemann, 1978). Saline has a similar effect to faeces when instilled into defunctioned colon (TVF) but the reason for this is unclear. (Clarke (1977) has found an equally unexplained tropic effect of saline in isolated loops of small bowel: mechanical stimulation seems the most likely explanation for these findings.

The presence of a normal physiological bacterial flora may be crucial to the maintenance of normal mucosal mass. Certainly, hypoplasia is found in the ileum of germfree rats (Abrams et al, 1962) and oral metronidazole, given alone, reduces both colorectal crypt depth and CCPR (Experiments D,E). Non-specific colonic hyperplasia can be induced in rats by infection with Citrobacter freundii (Barthold and Beck, 1980). The main influence of bacteria in colonic cell kinetics may depend on their relationship with other faecal constituents in particular bile acids.

Bile Acids

Surgical shortening of the gut brings the duodenal papilla closer to bowel distal to the line of resection where the mucosa could be exposed to higher than normal concentrations of bile and pancreatic juice. Pancreaticobiliary secretions might, therefore, have a significant influence on the control of intestinal adaptation. Whether they are directly tropic or

trophic to intestinal mucosa remains controversial (Chapter 8). Primary bile acids can be converted by colonic bacteria (particularly anaerobes) to secondary forms which may, in turn, undergo further bacterial degradation (Aries et al, 1969; Hill, 1979). The effect of primary bile acids alone on mucosal cell kinetics is unknown, but instillation of a secondary form (sodium deoxycholate SDC), into functioning rectum causes significant increases in colorectal crypt depth and crypt cell production rate (Experiments D,E). This hyperplasia was unaffected by metronidazole which, given alone, reduced both these parameters. Faecal anaerobes, therefore, are probably unnecessary for the tropic action of SDC. It is unlikely that SDC is directly nutritive to the mucosal cell; it may stimulate hyperplasia by causing chronic irritation and mucosal inflammation. However no evidence of an inflammatory response was seen on any of the histological sections prepared for estimation of crypt depth (Experiment D). As irrigations were stopped 3 weeks before sacrifice in this study, inflammation could have resolved while its effect on cell proliferation remained detectable. In man, colonic mucosal cell turnover seems to be increased in ulcerative colitis, even in quiescent phases of the disease (Allan et al, 1984).

By contrast, SDC did not reverse the mucosal hypoplasia, evidenced by reduced crypt depth, in defunctioned colon (TVF). Clearly for SDC to exert its tropic effect the presence of some other faecal constituent is required. As the population of faecal anaerobes seems unimportant, bulk or its mechanical stimulus might be crucial in SDC-induced hyperplasia.

PART II

Experimental Carcinogenesis

The Model

Points of similarity and differences between human colorectal cancers and azoxymethane-induced intestinal cancers in the rat have been discussed (Chapter 3). Experimental data presented in this thesis confirm the value of this model in aetiological studies of intestinal cancer.

Hyperplasia and Neoplasia

The results of Experiments A, B and D support the hypothesis that the susceptibility of rat gut to carcinogenesis is related to its mucosal proliferative state. Tumour yield in the large bowel is enhanced by manipulations which induce colonic hyperplasia, such as small bowel resection or bypass or direct exposure of the mucosa to a secondary bile acid solution (Experiments A,D). Conversely, colonic defunction as a TVF results in atrophy and reduced carcinogenesis in the isolated loop (Experiment B). Other workers have noted the tumour-promoting effects of surgically-induced adaptive hyperplasia (Oscarson et al, 1979; Hart et al, 1982; Williamson, 1982a; Scudamore and Freeman, 1983). The protective effect of defunction on colonic carcinogenesis induced by azoxymethane and related compounds, which reach the mucosa largely via the bloodstream, is also well documented (Wittig et al, 1971; Campbell et al, 1975). Carcinogenesis in response to contact carcinogens such as 2,4-dimethyl-amino-biphenyl is abolished completely distal to a defunctioning colostomy (Cleveland et al, 1967; Navarette and Spjut, 1967). In contrast to the situation in defunctioned or bypassed ileum (Experiment A) (Williamson and Bauer, 1978; Williamson et al, 1980b), there is clear evidence that isolation

from the faecal stream results in profound mucosal hypoplasia (Experiments B,C) (Tilson et al, 1976; Terpstra et al, 1981). Colonic hypoplasia following transfer to a liquid elemental diet also depresses large bowel carcinogenesis (Castleden, 1977; Janne et al, 1977).

The relationship between the mucosal proliferative state and susceptibility to carcinogenesis might be direct or indirect. Hyperplasia appears to be an essential prerequisite of chemical carcinogenesis (Cayama et al, 1978): both azoxymethane and dimethylhydrazine induce colorectal hyperplasia prior to neoplasia (Experiment A) (Williamson et al, 1978d; Oscarson et al, 1979). In mice, carcinogenesis in response to dimethylhydrazine is reduced when polyamine biosynthesis (which accompanies cell proliferation) is blocked by a specific ornithine decarboxylase inhibitor (Kingsnorth et al, 1983). It seems likely that the tropic action of the carcinogen is supplemented by adaptive hyperplasia and reversed or offset in atrophic or defunctioned bowel.

Hyperplasia could predispose to neoplasia by increasing the number of cells at risk of malignant transformation. Analogous mechanisms might explain increased carcinogenesis in mouse colon rendered hyperplastic by infection with Citrobacter freundii (Barthold and Beck, 1980) and in regenerating rat liver after partial hepatectomy (Pound and McGuire, 1978). Alternatively hyperplasia could facilitate rapid division of abnormal cells and the establishment of a clone. Crypt cell proliferation is thought to originate in a small population of slowly-cycling stem cells situated near the crypt base (Chang and Leblond, 1971; Cheng and Leblond, 1974). One daughter cell from each stem cell division then moves up into the proliferative compartment, where further division and differentiation take place before maturation and upward migration towards the mucosal surface.

Cairns (1975) has suggested that stem cells selectively "export" damaged or abnormal DNA in the daughter cell that migrates up the crypt column and which is discarded into the bowel lumen within a few days. Thus, potentially dangerous or premalignant chromosomal aberrations are quickly eliminated, while the remaining daughter (the new stem cell) retains undamaged DNA: the "immortal strain". It is unclear whether the rate of stem cell division is accelerated in mucosal hyperplasia, but is conceivable that this genetic "housekeeping" mechanism might become less efficient if it were. Abnormal stem cells might then be retained in the crypt to divide and establish clones and subsequently neoplasms. It will be interesting to see whether agents such as hydroxyurea, known to stimulate stem cell turnover (Al-Dewachi et al, 1977) enhance chemical carcinogenesis.

Creation and subsequent closure of a defunctioning transverse colostomy enhances carcinogenesis in the distal colorectum even when the carcinogen is commenced immediately after colostomy closure when the colon is still severely hypoplastic (Terpstra et al, 1981). This observation suggests that the number of proliferating cells is more important than the total number of epithelial cells.

Results in Experiment A support the suggestion of Hart and coworkers (1982) that hyperplasia induced by distal ileal resection does not actually increase tumour yield but simply reduces the latent period between exposure to the carcinogen and the subsequent development of macroscopic tumours. In other words, tumours grow faster in a hyperproliferative environment. In this experiment the carcinogen was given after the surgical operation. As adaptation has been detected within 48 hours following resection and slightly later after bypass, initiation and promotion were probably occurring almost simultaneously. Other studies have shown that surgically-induced

hyperplasia also promotes carcinogenesis when operation is delayed until after the course of carcinogen is complete (Williamson et al, 1978d; Bristol et al, 1982b). Indeed the timing of surgery in relation to the administration of carcinogen seems to have little effect on subsequent tumour yield (Williamson et al, 1979, 1982).

Luminal Factors

A less likely explanation for cancer promotion by adaptive hyperplasia, is that the susceptibility of intestinal epithelium to malignant transformation is influenced by the same luminal factors that govern adaptation. This argument suggests an indirect relationship between hyperplasia and neoplasia. Certainly, after all operations known to promote carcinogenesis the colorectal mucosa could be exposed to abnormal amounts of luminal nutrients and endogenous secretions, some of which might be carcinogenic in the colon. However, rapid functional enteric adaptation could presumably reduce this effect. The same procedures bring the duodenal papilla closer to the colonic mucosa, thus possibly increasing the faecal concentration of bile acids strongly implicated as endogenous cocarcinogens (Reddy, 1981; Thompson, 1982). However, Williamson and coworkers (1979) failed to detect any change in the nature or amount of bile acids excreted in rat faeces after pancreaticobiliary diversion (PBD). Again early ileal adaptation to jejunectomy and PBD may increase bile acid absorption in the distal small bowel and so prevent an increased bile acid load to the colon. Luminal changes can scarcely account for increased numbers of tumours proximal to the site of enterectomy, which is more readily explained by systemically-mediated adaptive hyperplasia (Williamson et al, 1980a; Williamson and Malt, 1980). Now that a specific ornithine

decarboxylase inhibitor is available which blocks polyamine synthesis and hence cellular proliferation, it should be possible to determine whether surgical shortening of the gut enhances carcinogenesis even in the absence of adaptive hyperplasia. Similarly, the tumour-promoting potential of non-surgical stimuli to adaptation such as cold acclimatisation (Jacobs and Dowling, 1982) and intermittent starvation (Fabry and Kujalova, 1960) merits investigation.

The many dietary and endogenous luminal constituents with potential roles as promoters or inhibitors of colorectal cancer are discussed in Chapter 4. In this thesis, three faecal constituents: bulk, bile acids and bacteria, have received further attention. Faecal bulk depends on its fibre content and its role in carcinogenesis seems complex. In its absence (following defunction or elemental diet), the colon atrophies and carcinogenesis is depressed (Experiment B) (Wittig et al, 1971; Campbell et al, 1975; Castleden, 1977). Yet the converse is not true. Increased faecal bulk is not known to stimulate hyperplasia, and rats given diets containing moderate amounts of fibre develop fewer chemically-induced tumours than rats on semisynthetic isocaloric fibre-free diets (Wilson et al, 1977; Freeman et al, 1978b). In man the bulking action of dietary fibre may have a protective effect against large bowel cancer by reducing exposure of the colonic mucosa to putative faecal carcinogens. This possible protective effect might explain why irrigation of a TVF with the faecal fluid (Experiment B) still enhanced carcinogenesis although the bulk of the irrigant had been substantially reduced by filtration and dilution. The relative lack of bulk may have facilitated cocarcinogenic activity in other faecal constituents. Mechanical stimulus alone seems important in colonic carcinogenesis, since saline irrigation enhanced TVF tumour yield to the same extent as the faecal irrigant.

Bile Acids

The epidemiological and experimental evidence implicating bile acids as major cocarcinogens is reviewed in Chapter 8. Results in Experiment D confirm that direct exposure of the colorectal mucosa to a secondary bile acid solution enhances chemical carcinogenesis. Using the contact carcinogen N-methyl-N'-nitro-N-nitrosoguanidine, Narisawa (1974) and Reddy and coworkers (1977b) showed that carcinogenesis was enhanced by intrarectal instillation of bile acids. Our results show that intrarectal bile acids also promote the carcinogenic effect of the parenterally-administered, blood-borne, azoxymethane.

It is tempting to attribute the tumour-promoting action of sodium deoxycholate (SDC) to its tropic effect on the epithelium, and there is accumulating evidence to support this hypothesis. Despite the reservations of some workers (Miazza et al, 1982) it is probable that pancreaticobiliary secretions are tropic to intestinal mucosa (Altmann, 1971; Williamson et al, 1978c), which may account for the increase in large bowel tumours following pancreaticobiliary diversion to mid-small bowel (Chomchai et al, 1974; Williamson et al, 1979). Deoxycholate instilled into functioning large bowel clearly increased distal colorectal crypt depths and CCPR while trebling tumour yields (Experiments D,E). An analogous hypothesis links bile acids to pancreatic carcinogenesis. Pancreatic duct hyperplasia has been found in association with cancer, and irrigation of canine pancreatic ducts with deoxycholate induces marked epithelial hyperplasia and atypia (O'Leary et al, 1984).

Bile acids could therefore promote neoplasia by simply increasing mucosal cell turnover and thus the number of cells at risk of malignant transformation, but they may also influence carcinogenesis by other

mechanisms. They might directly damage the epithelial cell; lithocholic acid can cause DNA strand breaks in cultured cells (Kulkarni et al, 1982). Receptors to deoxycholic acid have been detected in human colonic cancers, suggesting that bile acids could be selectively tropic to tumour cells (Summerton et al, 1982).

Since the protective effect of metronidazole on SDC-tumour promotion is not associated with a corresponding depression in SDC-induced hyperplasia, anaerobic bacteria may play an important part in mediating the cocarcinogenic action of bile acids, as Hill (1975) has suggested. We were unable to detect any decrease in the population of faecal anaerobes following 10 weeks of oral metronidazole, probably for the reasons outlined in Experiment E, but the metabolic activity of the anaerobes is probably more important in this context than their actual numbers (Reddy et al, 1980). Bacteria are clearly not essential, however, as both primary and secondary bile acids promote carcinogenesis in germ-free rats (Reddy et al, 1976c, 1977b; Reddy and Watanabe, 1979).

Interestingly, SDC was not cocarcinogenic in defunctioned colon (TVF) (Experiment B). This may reflect either its failure to reverse the profound defunction atrophy or a reduction or alteration in the colonic bacterial flora. By contrast, the faecal irrigant which partly restored normal carcinogenesis to the TVF, though lacking in bulk, probably contained both bile acids and bacteria in near normal amounts. Another possible explanation for the lack of response to SDC in this model is the detergent action of the bile acid solution. TVFs in other groups frequently became blocked with plugs of mucus and cellular debris which could be difficult to dislodge. This material could have had a slight stimulatory effect on the epithelium and thus prevented more profound atrophy in the TVFs than was

found in Experiments B and C. Regular flushing with the SDC solution thoroughly cleansed the TVF and prevented mucous plugs forming in this group. The SDC itself might have exerted a tropic effect similar to that of the accumulated debris but of insufficient intensity to stimulate neoplasia. Alternatively the presence of some other faecal constituent may be necessary for bile acids to exert their tropic and cocarcinogenic effects. Apart from bacteria, ammonia and other products of protein and urea degradation have been suggested for this role (Wynder and Reddy, 1973).

Further studies on bile acids and colorectal carcinogenesis are indicated. In this work, SDC promoted tumour development when given after the carcinogen. Reversing this order would reveal whether the large bowel mucosa can be "primed" by exposure to the bile acid before the carcinogen is administered. More detailed bacteriological studies would yield more useful information on the relationships between bacteria, bile acids and carcinogenesis.

Caecal Resistance to Carcinogenesis

Although the luminal environment has an important regulatory role in adaptation and carcinogenesis in susceptible bowel, it appears to play little part in governing the resistance of some segments to cancer.

Like its human counterpart the appendix, rat caecum is an uncommon site of carcinogenesis (Experiment F). Exposure to the faecal stream of the highly susceptible distal colon does not overcome its innate resistance. Likewise the distal small bowel is extremely resistant to cancer in animals and man (Williamson, 1982a). Only rats with direct irradiation of exteriorised bowel (Coop et al, 1974) and patients with longstanding Crohn's ileitis seem to be at any appreciable risk of adenocarcinoma of the ileum

(Hoffman et al, 1977). Transposition of ileal segment to right or left colon in the rat fails to render them susceptible to cancer (Celik et al, 1981) whereas colonic segments transposed to a small bowel environment retain their sensitivity to azoxymethane and dimethylhydrazine (Gennaro et al, 1973).

Luminal factors seem, therefore, less important in maintaining the innate resistance of these segments to cancer than the inherent characteristics of the mucosa. Furthermore this reduced susceptibility appears unrelated to the mucosal proliferative state. Both ileum and, to a lesser extent, the caecum respond to the adaptive effects of various surgical stimuli. Intense ileal hyperplasia follows jejunal resection, pancreaticobiliary diversion or subtotal colectomy, but virtually no ileal tumours develop whether operation precedes or follows the administration of carcinogen (Williamson, 1982a). Although caecal transposition produced only minor fluctuations in CCPR (Experiment F), ileal resection/bypass and colonic bypass stimulated caecal growths without increasing caecal tumour yield (Experiments A,B).

Local epithelial defence mechanisms seem likely to be crucial in the resistance of the two segments to neoplasia. In the ileum, mucosal enzymes may provide a metabolic barrier to ingested carcinogens or those elaborated from endogenous precursors such as bile acids (Wattenberg, 1971). Alternatively, carcinogenesis might be inhibited by the absence of specific enzymes involved in the local production of the ultimate carcinogen (Williamson, 1982a).

The ileum has a high content of lymphoid tissue and a substantial output of IgA: observations which imply an immunological defence mechanism. This inference is supported by the occurrence of primary or

metastatic distal small bowel cancer in immunodepressed patients such as renal transplant recipients, patients on corticosteroids for advanced breast cancer, and those with malignant melanoma (Lowenfels, 1973).

Similar biochemical or immune defence mechanisms might operate in the caecum where they seem slightly less effective. Unlike the ileum, the caecum is susceptible to the cancer-promoting effect of the chronic inflammation caused by insertion of a silk stitch (Pozharisski, 1975) or caecotomy and resuture (Experiment F). Indeed the only caecal tumours to develop in this experiment arose at the resutured caecotomy in 3 control animals.

Although the influence of putative local protective factors is most clearly manifest in insusceptible segments, they might have more widespread effects. Luminal contents passing through the ileum and caecum might be "treated" in some way rendering them less carcinogenic. This hypothesis could explain several experimental findings. (i) Subtotal colectomy (including caecectomy) and ileorectal anastomosis enhances rectal carcinogenesis (Williamson et al, 1980b), whereas colonic bypass (as a TVF) with caecorectal anastomosis fails to enhance rectal tumour yield, despite accelerating rectal CCPR (Experiment B). (ii) In control groups the incidence of tumours is low in the proximal colon but increases with distance from the caecum (Experiments A,B,D). Perhaps potential luminal precarcinogens are gradually reactivated by colonic bacteria. (iii) The DNA and protein contents of the right colonic mucosa are increased by left hemicolectomy but this moderate hyperplasia is not associated with increased susceptibility to carcinogenesis (Williamson et al, 1982). Since right hemicolectomy (including the caecum) has no effect on distal colonic tumour yield it is interesting to speculate on the effect of right

hemicolectomy with the caecum retained. Carcinogenesis in the distal colon could conceivably be reduced by its proximity to the caecum.

Suture-line Cancer

Intestinal anastomotic tumours are a common feature in both experimental animals (Williamson, 1982a; Williamson et al, 1982) and man (Ryall, 1907; Cohn, 1967). They were a frequent finding in these experiments. Apart from the resutured caecotomy discussed above, they occurred at a variety of anastomoses: ileocolic (Experiment F), colocaecal (Experiment F), caecorectal (Experiments B,F) and colorectal (Experiment B). Most anastomoses seem susceptible: duodenal (Williamson et al, 1979), duodenoileal (Williamson et al, 1979), jejunoileal (Williamson et al, 1980b), colocolic (Gennaro et al, 1973), jejunorectal (Celik et al, 1981) and ileorectal (Williamson et al, 1980b). Tumours do not occur at ileoileal (Experiment A) (Oscarson et al, 1979), ileocaecal (Experiment A) or colocolic anastomoses close to the caecum (Experiment B) (Gennaro et al, 1973). In man suture-line cancer is much more common at colocolic than at ileocolic anastomoses (Wright et al, 1969a).

For experimental suture-line tumours to develop it generally seems necessary for at least one bowel end to be a segment that is usually susceptible to the carcinogen (Williamson et al, 1982). Anastomoses involving only the ileum, caecum or proximal ascending colon are therefore usually spared.

In animals, suture lines are sites of chronic inflammation and hyperplasia (Pozharrski, 1975; Williamson, 1978) presumably as a result of the presence of the suture material, which could account for their susceptibility to cancer. Similar changes might explain anastomotic

recurrence following potentially curative colonic resection for carcinoma in man, but inadequate resection and retrograde lymphatic spread may be equally important factors. Whether tumour cells can implant at anastomoses at the time of operation is uncertain. Despite earlier reports to the contrary (Rosenberg, 1979) tumour cells shed into the bowel lumen do appear viable (Umpleby et al, 1984) and could conceivably be capable of implantation. In rats suture-line cancers develop whether the operation is performed before or after the administration of carcinogen (Williamson et al, 1982), and implantation is an unlikely explanation for the development of neoplasia in adults who have undergone ureterosigmoidostomy in childhood (Leadbetter et al, 1979; Spence et al, 1979).

Any slight narrowing or stricturing at an anastomosis could result in repeated trauma to the mucosa as faeces is propelled over it. Trauma can predispose to rectal carcinoma both in mice with chronic rectal prolapse (Wells et al, 1938) and in passive male homosexuals (Cooper et al, 1979). Likewise repeated abrasion of the skin promotes carcinogenesis (Argyris, 1980). Trauma might also explain the development of stomal cancer (Experiment B), which has even been found in the absence of carcinogen in one particular strain of Wistar rat (Winkler et al, 1982).

Clinical Relevance

This work supports the hypothesis that factors that stimulate colorectal cell proliferation predispose to carcinogenesis. It is always difficult to extrapolate from small laboratory animals to man, but our observations may have disturbing relevance for a variety of patients.

The increased risk of colorectal cancer in patients with longstanding ulcerative proctocolitis is well established (Lennard Jones et al, 1977; Van

Heerden and Beart, 1980). There is increasing evidence that the disease is associated with increased mucosal cell proliferation in both active and quiescent stages, which may explain its predisposition to cancer (Eastwood and Trier, 1973; Allan et al, 1984). An increased risk of rectal cancer also follows pelvic irradiation for benign or malignant gynaecological conditions (Martins et al, 1980). X-rays cause an initial hyperplastic repair phase in man (Williamson, 1978) and induce rectal cancers in mice (Hirose et al, 1977).

There have been occasional reports of carcinoma in young patients with shortened gut (Pentlow, 1976; Sachatello, 1979), but on the other hand subtotal colectomy and ileorectal anastomosis has occasionally led to regression of rectal polyps (Hubbard, 1957; Cole and Holden, 1959; Shepherd, 1971; Bussey, 1978). Long-standing Crohn's disease probably carries an increased risk of cancer of the large bowel and almost certainly of the small bowel (Hoffman et al, 1977; Greenstein et al, 1980; Gyde et al, 1980). Distal enterectomy is commonly performed in the management of this condition, but there is no indication that it intensifies the risk of colorectal cancer. Indeed patients nearly always develop cancer in an inflamed segment rather than in adjacent bowel that may have adapted to fulfil the function of a diseased or missing segment. One third of small bowel Crohn's carcinomas are found in bowel that is partly defunctioned, usually by ileotransverse colostomy (Greenstein et al, 1980). Nevertheless, patients undergoing extensive ileal resection, who are often young, could live long enough to develop postoperative cancer, if intestinal shortening promoted neoplasia in man as it does in rats.

In Crohn's disease the indications for operation are usually clearcut, and resection(or bypass) is unavoidable. Theoretical objection to such

procedures on the grounds of a possible increased risk of bowel cancer in the distant future seem clinically irrelevant. By contrast the indications for distal ileal bypass carried out to control hyperlipidaemias (Buchwald et al, 1974) are less compelling, as alternative drug treatments are available. The tumour promoting effects of experimental distal ileal bypass should be borne in mind when this operation is being considered in otherwise healthy young patients.

As bile acids are potent intestinal cocarcinogens in rodents, it seems reasonable to suspect that they might have a similar effect in man. Epidemiological studies provide strong circumstantial evidence to support this contention, and careful monitoring of several groups of patients is indicated (Sachatello et al, 1980). The alleged link between cholecystectomy and right colonic cancer in women (Werner et al, 1977; Alley and Lee, 1983) was not confirmed in a large retrospective study of colorectal cancer carried out recently in Bristol (Umpleby et al, 1984). Furthermore, cholecystectomy does not promote chemical carcinogenesis in mice (Schattenkerk et al, 1980). Nevertheless loss of the biliary reservoir might result in a greater turnover of bile acids and explain the higher proportion of degraded forms reaching the large bowel (Pomare and Heaton, 1973; Hepner et al, 1974). Alternatively cholelithiasis and colorectal cancer might share common aetiological factors despite differing sex incidences. While the long term effect of cholecystectomy on colorectal cancer risk remains unclear, patients should perhaps be considered to be at increased risk after this operation and carefully followed-up. Ideally they should be regularly screened for large bowel cancer at frequent intervals. Bile acid therapy as an alternative to cholecystectomy may prove to have a much more appreciable and potentially dangerous effect on the composition

of faecal bile acids. Similarly, patients on hypocholesterolaemic therapy (cholestyramine increases faecal bile acid content), and oestrogen therapy (the contraceptive pill alters bile acid metabolism) should be submitted to more intensive surveillance than the rest of the population.

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APPENDIX
PUBLICATIONS RELATING TO THIS THESIS

Adaptation and carcinogenesis in defunctioned rat colon: Divergent effects of faeces and bile acids

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Summary Because the composition of faeces modulates colorectal carcinogenesis, promotional effects of the secondary bile salt sodium deoxycholate (SDC) were compared with those of dilute homogenised faeces (12.5% w/v) or saline alone in rat colon isolated from the faecal stream as a Thiry-Vella fistula (TVF). Each fluid was used to irrigate a group of TVFs 3 times per week for 12 weeks. Other rats had TVF without irrigation or colonic transection and reanastomosis (sham TVF). Operations followed a 6-week course of azoxymethane injections. At sacrifice 15 weeks postoperatively crypt depth and tumour yield were reduced to the same extent in both the non-irrigated TVFs and the SDC-irrigated TVFs, when compared to shams. Irrigation with faeces and saline completely restored crypt depth and partly restored tumour yields to the levels in shams. Tumours were smaller in the SDC group than in the other 4 groups. While tumours developed mainly in the left colon of shams, there was significantly more even distribution in the TVFs. Exclusion of the colon from the faecal stream leads to mucosal hypoplasia and impaired carcinogenesis. Irrigation with faeces or saline partly reverses these changes. Deoxycholate has no such effect and clearly is not co-carcinogenic in this model.

Increased crypt-cell proliferation leads to greater numbers of intestinal tumours in susceptible animals, including man. Thus surgical shortening of the gut in rats and inflammatory bowel disease in man promote colorectal carcinogenesis (Williamson, 1982a). As a corollary, environmental changes that depress cell turnover might be expected to decrease carcinogenesis in the affected bowel. Starvation and interruption of normal anatomical continuity are the two most effective methods of causing intestinal hypoplasia (Steiner *et al.*, 1968; Terpstra *et al.*, 1981). Individuals who are <80% of ideal body weight may enjoy some protection against colon cancer (Lew & Garfinkel, 1979). Defunctioning proximal colostomy greatly reduces the number of cancers in the distal colon of rats exposed to azoxymethane or dimethylhydrazine, agents that reach the mucosa largely via the bloodstream (Wittig *et al.*, 1971; Campbell *et al.*, 1975). With the contact carcinogen 2,4-dimethyl-4-aminobiphenyl, which is delivered via the intestinal lumen, colostomy protects the distal colon completely (Cleveland *et al.*, 1967; Navarrete & Spjut, 1967).

Not only the presence of faeces but its precise composition affect the development of colorectal neoplasia. Diets enriched with fat and depleted of fibre increase both faecal excretion of bile acids and the number of colonic bacteria capable of further degrading secondary bile acids to potential pro-carcinogens (Reddy & Wynder, 1973; Hill, 1974). Moreover, administration of cholic acid by mouth

and of deoxycholic acid per rectum promote the development of experimental tumours (Reddy *et al.*, 1976; Cohen *et al.*, 1980).

The present experiments have used loops of rat colon isolated as a Thiry-Vella fistula (TVF) to compare the local effects of saline, deoxycholic acid and dilute faeces in altering the number of tumours induced by azoxymethane. Only saline and faeces seem to reverse the atrophic and protective effects of colonic defunction.

Materials and methods

Experimental animals

One hundred and five male Sprague-Dawley rats (Olac SD, Bicester, Oxon, England) weighing 120-150 g were received into the animal house 1 week before the start of the experiment and were allocated to 1 of 5 groups (Figure 1). They were fed standard rat chow (Oxoid Breeding Diet; HC Styles & Co Ltd, Bewdley, Worcs.) and water *ad libitum*. Animal quarters were lit in alternate 12-hourly cycles. Rats were weighed weekly throughout the experiment. All animals received weekly intraperitoneal injections of azoxymethane (Ash Stevens Inc, Detroit, Michigan, USA) 15 mg kg⁻¹ for 6 weeks. Each rat was submitted to operation 7-12 days after the last injection of carcinogen.

Surgical operations

At laparotomy the right colon was transected 1 cm distal to its origin from the caecum, and the left colon was transected just above the pelvic brim. In

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Operations were performed under light ether anaesthesia. Continuous 6/0 silk sutures were used for intestinal anastomoses. The proximal and distal stomas were secured with interrupted 6/0 chromic catgut sutures approximating mucosa to skin. At the end of each operation 0.25 mg Vitamin K was administered i.m. to prevent the troublesome postoperative bleeding encountered in young Sprague-Dawley rats in earlier experiments (Bristol *et al.*, 1982b). Rats in Group 1 (sham TVF) and Group 2 (TVF alone) had no further manipulations between operation and death.

Irrigation of the TVF

One week after operation the TVF was irrigated for the first time in Groups 3–5 (Figure 1). Irrigations were carried out using 5 ml plastic syringes, the hubs of which could be fitted comfortably into either stoma of rats suitably restrained by an experienced handler. An initial bolus of 5 ml N saline was administered to rats in all three groups to clear the fistula of mucus and retained faeces. It was not possible to carry out all irrigations from the same side. TVF contents (retained mucus and subsequently tumours) often produced a "ball valve" effect, making it necessary to instill volumes from each end alternately.

Once patency had been demonstrated by the appearance of the irrigant at the opposite stoma, a second bolus of irrigant was administered. Rats in Group 3 (TVF+saline) received a further 5 ml N saline. Group 4 rats (TVF+SDC) received 5 ml 0.12 M sodium deoxycholate, prepared by dissolving 50 g sodium deoxycholate (Sigma Chemical Co., St Louis, USA) in 1 litre N saline; each 5 ml aliquot contained 0.25 g (600 μ mol) Na deoxycholate. Group 5 rats (TVF+faeces) received 5 ml of a 12.5% w/v suspension of rat faeces in N saline prepared by collecting and homogenising faeces from rats not receiving carcinogen. The suspension was filtered through surgical gauze to remove the larger fibres and enable delivery into the fistulas via a syringe. Each irrigant solution was administered 3 times a week (Monday, Wednesday and Friday) for 12 weeks, beginning at week 7 (Figure 1).

Autopsy specimens

Rats were regularly examined for evidence of tumour development and were killed when moribund or at the end of 22 weeks. At autopsy the entire intestinal tract was excised. The following segments were thoroughly flushed with cold saline to remove all content: duodenum, jejunoleum, caecum, colon between anastomoses (or TVF) and rectum. The length of each segment was determined

by suspension with a 9.5 g weight against a ruler, and the surface area of the caecum was estimated as previously described (Williamson *et al.*, 1980a). The weights of the liver, kidneys and spleen were also recorded. Intestinal segments were opened, and the number, size and position of all tumours were recorded. The tumours were excised, and the remaining bowel was blotted dry and weighed. All tumours were fixed in 10% formalin prior to histological processing. Subsequently 5 μ m sections were prepared for staining with haematoxylin and eosin.

A 1-cm specimen of colon was excised from the middle of each TVF or from the mid transverse colon in shams, and similar histological sections were prepared. The mean crypt depth was estimated by ocular micrometry of 10 perfectly-sectioned crypts per slide.

Statistics

Student's *t*-test was used for statistical analysis of the data.

Results

Mortality rate

Nine rats (8.5%) died before the end of the first postoperative week, from either haemorrhage or anaesthetic overdose. Most subsequent deaths resulted from rupture of the TVF during irrigation (9 rats), caecal volvulus around the TVF (5), or strangulated intestinal hernia. (2). Three rats with suspected burst TVFs were re-explored immediately but without success. The yields of surviving animals at the end of the experiment are given in Figure 1.

Body weight

At the end of the first postoperative week the mean weight of the groups with a TVF was 5–24% lower than immediately before operation, while shams had regained their preoperative value. Thereafter, all rats gained weight steadily, but TVF rats remained a little lighter than the shams. At the end of the experiment, the mean weight of the TVF groups varied between 496 and 520 g, i.e. 91–96% of the weight of the shams (544 ± 16 g, sem: $P < 0.05$).

Intestinal adaptation

The mean length of the TVF in all 4 groups was 12.1 ± 0.2 cm (sem) and the mean weight was 2.1 ± 0.2 g. By contrast, the equivalent segment of colon between the anastomoses in shams was 20.0 ± 0.6 cm long and weighed 3.8 ± 0.2 g.

($P > 0.001$). Among the four groups of TVF rats the mean TVF length ranged from 11.4–12.9 cm and the TVF weight from 1.7–2.9 g, irrespective of irrigation. No significant differences were found between any of the groups in the weight of the duodenum, jejunum, caecum, liver, kidneys or spleen, nor in the surface area of the caecum or the length of the other intestinal segments.

The mean colonic crypt depth (Figure 3) in sham colons was $274 \mu\text{m} \pm 4$ (sem), compared with 242 ± 6 in the non-irrigated TVFs and 241 ± 6 in the SDC-irrigated TVFs ($P < 0.001$). In those TVFs irrigated with faeces and saline, crypt depth did not differ from that in the shams (280 ± 6 and 276 ± 7).

Intestinal tumours

All but 2–3 rats in each group developed one or more tumours in the isolated colon (TVF) or equivalent segment of functioning colon between anastomoses (in shams). The mean number of these tumours per rat in the 4 combined TVF groups (2.7) was 25% lower than the mean number in shams (3.6) (Table I). Including those tumours arising at a stoma or colonic anastomosis increased the numbers in both TVF rats (3.0) and shams (4.3) and increased the difference to 30% ($P < 0.05$). Tumour yields were lowest (64–67% of shams) in TVFs that were either not irrigated (TVF alone) or were irrigated with SDC ($P < 0.05$). In rats with saline or faecal irrigation yields were still only 79–

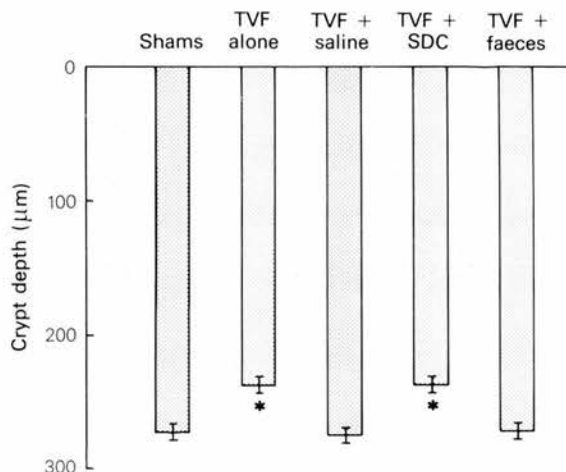


Figure 3 Colonic crypt depth (mean \pm sem). TVF = Thiry-Vella fistula. Significance: * $P < 0.001$ vs other three groups.

85% of those in shams, but these differences no longer attained statistical significance.

Substantial numbers of tumours arose in the duodenum, jejunum and rectum in each group of animals, but these numbers were not affected by creation or irrigation of a colonic TVF (Table I). In addition, one rat had a caecal tumour, one a gastric tumour and 5 had tumours of the external auditory canal. Carcinomatosis peritonei occurred in 11 rats.

The presence of a TVF altered the distribution of colonic tumours irrespective of irrigation (Figure 4).

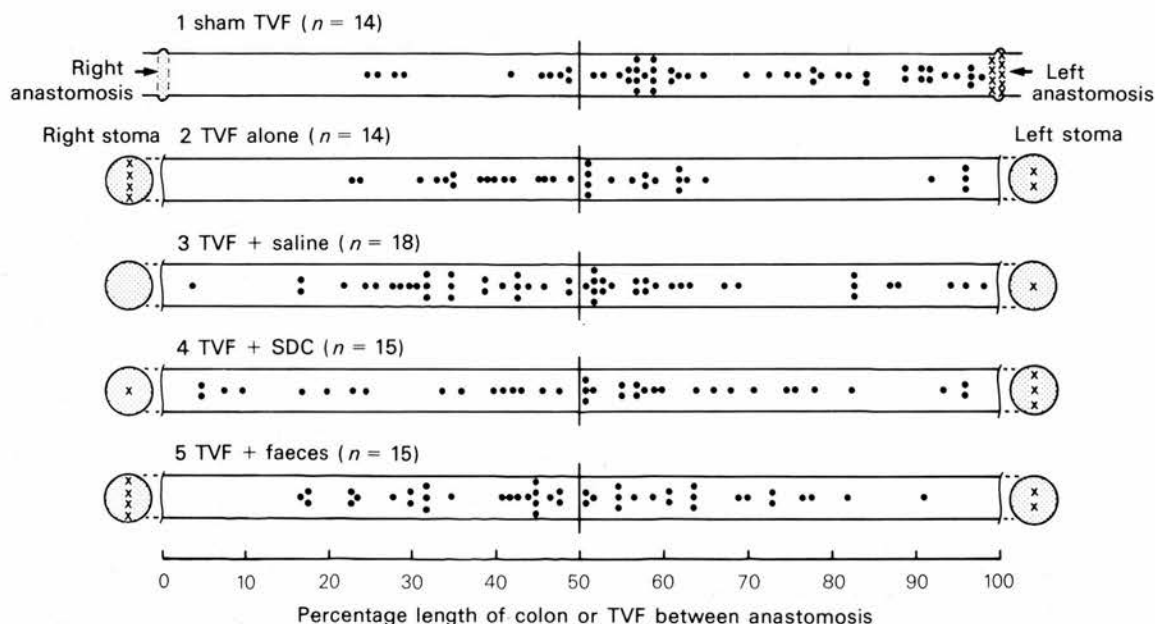


Figure 4 Distribution of tumours in Thiry-Vella fistulas (TVFs) or sham TVF. Each tumour in the TVF (or equivalent colon) is shown by a solid circle. Each tumour at the stoma or anastomosis is shown by a cross.

Table 1 Number of tumours per rat (mean \pm s.e.) in groups with and without a colonic TVF (Thiry-Vella fistula)

	1. <i>Sham TVF</i>	2. <i>TVF alone</i>	3. <i>TVF+saline</i>	4. <i>TVF+SDC</i>	5. <i>TVF+faeces</i>	2-5. <i>All TVFs</i>
Duodenum	2.2 \pm 0.4	2.2 \pm 0.3	2.6 \pm 0.5	2.1 \pm 0.4	1.3 \pm 0.4	2.1 \pm 0.4
Jejunioileum	0.5 \pm 0.2	0.2 \pm 0.1	0.4 \pm 0.2	0.1 \pm 0.1	0.3 \pm 0.3	0.3 \pm 0.1
Colon						
(less stomas/anastomoses)	3.6 \pm 0.5	2.4 \pm 0.4*	2.9 \pm 0.4	2.3 \pm 0.4	3.1 \pm 0.6	2.7 \pm 0.2
Stomas/anastomoses	0.6 \pm 0.3	0.1 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1	0.4 \pm 0.2	0.3 \pm 0.1
Total Colon	4.3 \pm 0.6	2.6 \pm 0.3*	3.2 \pm 0.4	2.6 \pm 0.4*	3.5 \pm 0.6	3.0 \pm 0.2*
Rectum	1.1 \pm 0.4	1.9 \pm 0.3	1.2 \pm 0.3	0.9 \pm 0.3	1.2 \pm 0.3	1.3 \pm 0.2

SDC=Sodium deoxycholate.

Significance *versus* shams.* = $P < 0.05$.

In sham rats 41 of 52 tumours (81%) arose within the distal half of the colon, whereas in TVF rats (groups 2-5) tumours were evenly distributed between the proximal (49%) and distal (51%) halves of the isolated colon. The yield of tumours in the proximal colon was 1.4 ± 0.1 in all TVFs compared with 0.7 ± 0.3 in the equivalent segment in shams ($P < 0.05$). No differences were observed between the non-irrigated and irrigated TVFs. Similarly, in TVF rats, tumours were distributed almost equally between the right stoma (9 tumours) and the left stoma (8), while in shams anastomotic tumours (9) were confined to the left colon.

The mean diameter of tumours found in the TVFs irrigated with sodiumdeoxycholate (2.6 ± 0.3 mm) was 41-51% less than the diameter of colonic tumours in the other 4 groups ($P < 0.01$). There were no other significant differences, however, and creating a TVF alone did not reduce tumour size.

Adenocarcinoma was the commonest histological type (80%), varying from carcinoma-in-situ to invasive cancer. Mucinous (colloid) adenocarcinomas (7%), characterised by the presence of "signet-ring" cells, were detected mostly in the duodenum and were all deeply invasive. Benign adenomas and hyperplastic polyps accounted for 13% of tumours detected. No differences in tumour histology were observed between the groups.

Discussion

Defunctioned colon develops fewer tumours than colon remaining in continuity. This finding can hardly be explained by the minor decrease in body weight found in the TVF rats, as we have shown in a previous study that 85% jejunioileal bypass enhances colonic carcinogenesis despite a 40%

reduction in body weight (Bristol *et al.*, 1982b). Our results are consistent with the findings of other workers (Wittig *et al.*, 1971; Campbell *et al.*, 1975) who have ascribed the protective effect of a defunctioning proximal colostomy to an altered population of bile acids and bacteria in the excluded distal colorectum. Our preliminary findings that neither resection nor bypass of the small intestine affect carcinogenesis in bypassed colon suggest that hormones are of secondary importance to a diversion of the faecal stream (Bristol *et al.*, 1982a). Rubio *et al.* (1980) in a similar experiment found no reduction in dimethylhydrazine-induced tumours in a small colonic TVF, but the number of rats was small and no control group was included.

Mucosal hypoplasia in the bypassed colon is indicated by shortening of the crypts and by the reduced weights and lengths of the TVFs; presumably this phenomenon accounts for the reduced susceptibility to carcinogenesis. There was a close correlation between tumour yield and crypt depth, each being reduced in the TVF (with or without deoxycholate) but indistinguishable from values in intact colon after irrigation of the TVF with saline or faeces. Other studies confirm that mucosal atrophy develops rapidly distal to the site of a proximal colostomy but is precisely reversed soon after continuity of the bowel is restored (Tilson *et al.*, 1976; Terpstra *et al.*, 1981).

Our results may shed some light on the role of various faecal constituents in colorectal carcinogenesis. Clearly, deoxycholate was not cocarcinogenic in this model, despite strong epidemiological and experimental evidence to support such a role for bile acids. According to Aries *et al.* (1969), their increased faecal excretion could well be the link between high-fat diets and susceptibility to cancer, especially after bacterial degradation to the secondary forms, deoxycholic

and lithocholic acid. Aromatisation of the sterol ring, possibly achieved by nuclear-dehydrogenating clostridia, could ultimately produce a carcinogen similar to cyclopentaphenanthrene (Hill *et al.*, 1971). Recently, receptors to deoxycholic acid have been identified in human colonic cancers (Summerton *et al.*, 1982). Rats fed 0.2% cholic acid in the diet produce more colonic tumours in response to a chemical carcinogen than rats on a normal diet (Cohen *et al.*, 1980). More bile acids are excreted in the faeces, mainly deoxycholate which is presumably the major co-carcinogen. Direct exposure of colorectal mucosa to primary and secondary bile acids instilled per rectum also promotes carcinogenesis (Narisawa *et al.*, 1974; Reddy *et al.*, 1977). Since the same phenomenon occurs in germ-free rats, albeit to a lesser extent, bacterial conversion of bile acids to cocarcinogens may not be essential (Reddy *et al.*, 1976, 1977, 1979).

Introduction of a secondary bile acid into isolated colon promoted neither hyperplasia nor neoplasia. Indeed deoxycholate irrigation actually reduced tumour size, suggesting a possible protective effect. These findings are at variance with the studies of Reddy and his colleagues showing enhanced carcinogenesis after the instillation of primary and secondary bile acids into the intact rectum of conventional and germ-free rats. There are certain methodological differences. In one experiment (Narisawa *et al.*, 1974) the bile acid was suspended in peanut oil, which might itself be carcinogenic, and appropriate controls were not included. In two others (Reddy *et al.*, 1976, 1977) a smaller total dose of deoxycholate was used (3 g versus 9 g), and in all three the direct-acting carcinogen N-methyl-N'-nitro-N-nitrosoguanidine was employed rather than parenteral dimethylhydrazine or azoxymethane. No doubt the intestinal microflora, possibly implicated in the cocarcinogenic role of bile acids, are both quantitatively and qualitatively different in a TVF as opposed to colorectum in continuity. Moreover, the absence of faeces might remove some constituent that is necessary for bile acids to exert their promoting effect or is itself an additional cocarcinogen. Ammonia and other products of protein and urea degradation have been suggested for this role (Wynder & Reddy, 1973), and the mechanical stimulus of faecal bulk could also be important.

Deoxycholate irrigation alone did not prevent the reduction in crypt depth found in the non-irrigated TVFs. Our previous experiments showing that bile and pancreatic juice could stimulate adaptive growth and carcinogenesis after surgical diversion involved distal gut that remained in continuity with the faecal stream (Williamson *et al.*, 1978, 1979).

Bile acid solutions are detergents, and this property could outweigh any cocarcinogenic role by cleansing the fistula of accumulated cellular debris and retained faeces that saline failed to dislodge.

Tumour yields in the TVFs irrigated with faeces were greater than in the non-irrigated TVFs but did not quite reach the level found in the shams. Faecal bulk is important in the maintenance of normal cell turnover (Williamson, 1982b) and was clearly diminished by the 8-fold dilution required to permit its delivery into the fistulas. The faecal irrigant failed to prevent the loss of weight and length in the TVF, but it did preserve normal crypt depth and it enhanced carcinogenesis. Mechanical stimulation may also be important, since saline irrigation had similar effects; saline alone can stimulate mucosal cell turnover in isolated loops of small bowel (Clarke, 1977). The relative lack of bulk in the irrigant may have facilitated cocarcinogenic activity in other faecal constituents, since it has been suggested that the bulking action of dietary fibre has a protective effect in human large-bowel cancer by reducing exposure of the colonic mucosa to putative faecal carcinogens (Heaton, 1977).

The anatomical redistribution of tumours in the TVF (regardless of irrigation) is of great interest. As the proximal colonic tumour yield was actually increased in the sequestered colon, the redistribution observed was not just the result of a relative reduction in the proportion of distal colonic tumours, which has been observed in low incidence populations and in animals receiving low-dose carcinogen (Lambert, 1982; Ross, 1982). The various substances administered did not affect the yield of proximal tumours. Since irrigation was not confined to one or other stoma, it is unlikely that the altered distribution of tumours was due to any "jet" effect. The normal left-sided preponderance of colonic tumours could reflect differences between the proximal and distal colon in the bulk and transit time of faeces, the population and activity of bacteria, and the rate of mucosal cell proliferation (Cooke *et al.*, 1982). Withdrawal of faeces would change all these conditions.

We have previously reported colostomy tumours in rats given azoxymethane (Terpstra *et al.*, 1981). Stomal cancers can develop spontaneously in Wistar rats not receiving carcinogen, probably owing to chronic irritation (Winkler, 1982). Similar susceptibilities of the ascending and descending colostomies mirrors the redistribution seen within the TVF and contrasts with the finding in shams that anastomotic tumours were confined to the left colon.

The failure of colonic bypass to increase carcinogenesis in the adjacent gut is not surprising, at least in the case of the ileum which is extremely resistant

to cancer (Williamson, 1982a). Although subtotal colectomy including caecectomy has a mild enhancing effect on rectal carcinogenesis, hemicolectomy has no such effect (Williamson *et al.*, 1980b, 1982c). The caecum was retained in continuity in the present experiment, and this may have prevented any promotion of rectal carcinogenesis. Moreover, bypass is probably a less potent

promoter of carcinogenesis than resection (Williamson *et al.*, 1980b).

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Relative effects of ileal resection and bypass on intestinal adaptation and carcinogenesis

Since ileal resection and ileal bypass are commonly performed in man and might stimulate colonic hyperplasia, their co-carcinogenic potential was explored in male Sprague-Dawley rats (n = 135). One week after 33 per cent distal small-bowel resection, 33 per cent distal small-bowel bypass or distal ileal transection (control), animals started a 6-week course of azoxymethane injections (total dose 90 mg/kg ip). Findings in rats killed at 20 and 25 weeks were similar: bypass produced a higher yield of colorectal tumours (4.0 ± 0.6 per rat: mean \pm s.e.) than controls (2.4 ± 0.4 ; $P < 0.05$), but resection caused maximal enhancement (5.2 ± 0.5 ; $P < 0.01$). In rats killed at 30 weeks, however, tumour yields were almost identical. Overall, resection increased colonic tumour yield by 55 per cent ($P < 0.02$) and bypass by 32 per cent. Stathmokinetic measurements of crypt cell production rate (CCPR) at 20 weeks showed similar increases after resection and bypass both in residual functioning small bowel (109-200 per cent; $P < 0.01$) and in colorectum (63-100 per cent; $P < 0.05$). At 30 weeks these adaptive effects persisted, despite an overall increase in CCPR with age. Loss of functioning ileum enhances experimental colorectal carcinogenesis principally by reducing the latent period for tumour development. Resection has a greater effect than bypass probably by producing earlier hyperplasia, though later adaptive effects are similar.

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Surgical shortening of the gut stimulates compensatory hyperplasia of the remaining bowel, which allows the organism to adapt to the loss of functioning tissue¹. Under certain circumstances, however, this physiological response may also predispose to carcinogenesis. In the rat, partial enterectomy produces sustained mucosal hyperplasia in the remaining small bowel distal to the line of resection and to a lesser extent in proximal small bowel and colon²⁻⁵. It also promotes chemically induced intestinal carcinogenesis⁶. Likewise, partial enteric bypass of sufficient extent will enhance carcinogenesis. Subtotal jejunioileal bypass results in structural adaptation of the colon and increased tumour yields, despite the loss of up to 40 per cent of body weight^{7,8}. Less extensive small-bowel bypass operations have a slower

but ultimately similar adaptive effect to resection; their effect on carcinogenesis is uncertain^{4,9}. These experimental findings could prove clinically relevant. Small-bowel resection is a common procedure, particularly in the management of Crohn's disease, subtotal jejunioileal bypass is often carried out for morbid obesity, and distal ileal bypass has been developed by Buchwald as an alternative to drug therapy in the management of hyperlipidaemias¹⁰. The present study was designed to compare the effects of resection and bypass of the distal small bowel on cell proliferation and carcinogenesis. How compensatory hyperplasia predisposes to carcinogenesis is not clear. The population of epithelial cells at risk of malignant transfor-

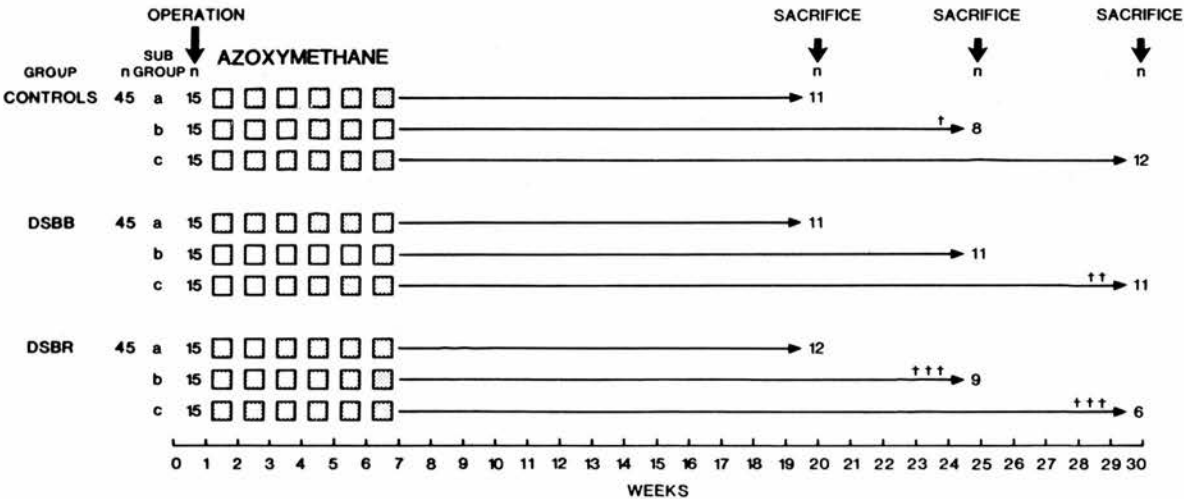


Figure 1 Experimental design. DSBB, distal small-bowel bypass; DSR, distal small-bowel resection; †, rats dying prematurely in which tumour yields were included in the overall data

mation might simply be increased or alternatively, cells which have undergone malignant change may be stimulated to divide more rapidly, thus reducing the delay between exposure to the carcinogen and the development of tumours⁴. In an attempt to answer this question, one-third of the animals in each operative group were sacrificed at 20, 25 and 30 weeks postoperatively.

Materials and methods

Experimental animals

One hundred and forty-five male Sprague-Dawley rats (Olac SD, Bicester, Oxon, England) weighing 100–125 g were received into the animal house 1 week before the start of the experiment and were allocated to one of three groups (Figure 1). They were fed standard rat chow (Oxoid Breeding Diet, H C Styles & Co Ltd, Bewdley, Worcs) and water *ad libitum*. Animal quarters were lit in alternate 12-hourly cycles. Rats were weighed weekly throughout the experiment.

Surgical operations

Operations were performed under light ether anaesthesia. At laparotomy the length of the small bowel from the ligament of Treitz to the ileocaecal valve was measured by gently stretching 5 cm segments against a ruler. In group 1 ($n=45$), transection and reanastomosis of the small bowel at the junction of its middle and distal thirds was combined with caecotomy and resuture as a control operation (Figure 2A). In group 2 ($n=45$), the distal 33 per cent of the small bowel was bypassed (DSBB), and intestinal continuity was restored by anastomosing the proximal two-thirds of the small bowel end-to-side to the caecum (Figure 2B). This operation is analogous to Buchwald's distal ileal bypass in man¹⁰. Rats in group 3 ($n=45$) underwent resection of the distal 33 per cent of the small bowel (DSBR) with end-to-side ileocaecostomy (Figure 2C). Continuous 6/0 silk sutures were used for intestinal anastomoses.

At the end of each operation 0.25 mg vitamin K was given by im injection to prevent the troublesome postoperative bleeding encountered in young Sprague-Dawley rats in earlier experiments⁷. One week later rats received the first of 6 weekly ip injections of azoxymethane 15 mg/kg. Rats in each operative group were further randomized to three subgroups, a, b and c. Groups 1a, 2a and 3a were to be sacrificed at 20 weeks postoperatively, groups b at 25 weeks and groups c at 30 weeks (Figure 1).

Autopsy specimens

Rats were regularly examined for evidence of tumour development and were killed at 20, 25 or 30 weeks, or earlier if moribund. At autopsy the entire intestinal tract was excised. The following segments were thoroughly flushed with cold saline to remove all content: duodenum, jejunum, ileum, caecum and colorectum. The length of each segment was determined by suspension with a

constant weight (9.5 g) against a ruler. The weights of the liver, kidneys and spleen were recorded. The tumours were excised, and the remaining bowel was blotted dry and weighed. All tumours were fixed in 10 per cent formalin before histological processing. Subsequently 5 μ m sections were prepared for staining with haematoxylin and eosin.

In addition, at 20 weeks and 30 weeks six rats from each subgroup received the stathmokinetic agent vincristine 1 mg/kg ip. Animals were then killed at half-hourly intervals from 30 to 180 min after vincristine. Autopsy was carried out as described above, but after excision of tumours the following intestinal segments were fixed in Carnoy's solution: proximal, middle and distal thirds of jejunum, caecum, proximal middle and distal thirds of colorectum. The distal jejunum specimen was from the bypassed loop after DSBB and was missing after DSBR. After 3–6 h these specimens were transferred to 70 per cent alcohol, in which they could be preserved indefinitely. Later, after staining with Schiff's reagent, ten individual crypts were isolated from each specimen by microdissection. The number of arrested metaphases counted in each crypt was plotted against time after vincristine. The crypt cell production rate (CCPR) was calculated from the slope of the least squares regression line which best fitted the data¹¹.

Statistics

Two CCPR regression lines were compared by pooling both sets of data and fitting a single regression line. The significance of the difference between their slopes was assessed by calculating an *F*-statistic, which indicated the degree to which the two separate lines explained the data more fully than the single combined regression line.

All other data were assessed for statistical significance using Student's *t* test.

Results

Mortality

Thirteen rats (9 per cent) died before the end of the first post-operative week, from haemorrhage or anaesthetic overdose. Another 22 animals died during the early part of the experiment from either mechanical obstruction at the anastomosis (12 rats), anastomotic leakage (6) or internal hernia (4). Between 6–8 weeks postoperatively, 4 rats (2 DSBB and 2 DSBR) died from gastrointestinal haemorrhage, possibly because of vitamin K malabsorption resulting from loss of functioning ileum¹². Further bleeding was prevented by a second im injection of vitamin K 0.25 mg administered to all animals during week 8. Four rats in the DSBR group died prematurely from cancer: 2 had tumours of the external auditory canal alone, and 2 had intestinal tumours at 14 and 16 weeks.

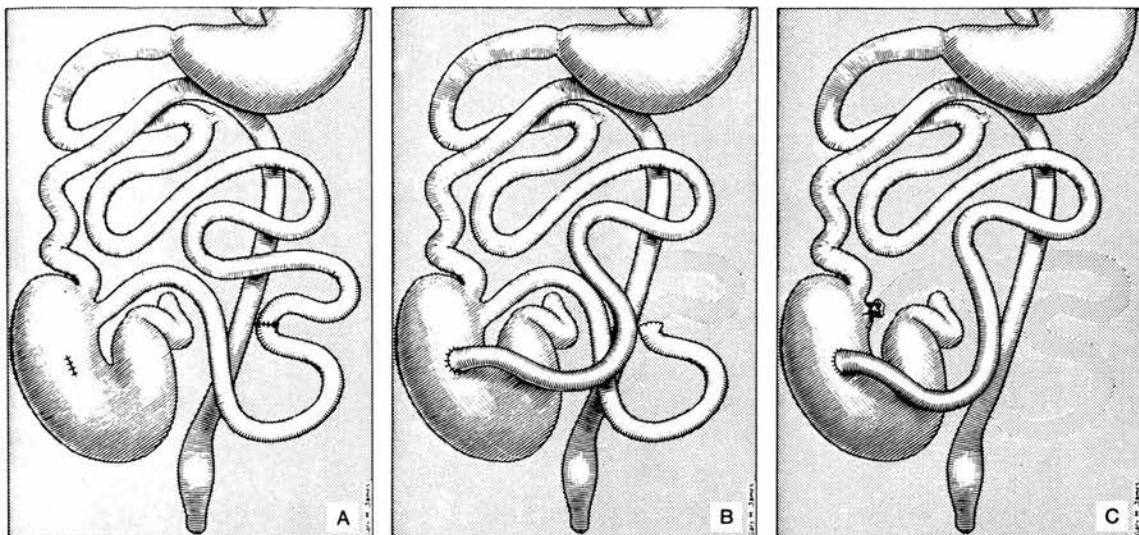


Figure 2 Operations: A, transection of the distal small-bowel, caecotomy and resuture (controls). B, 33 per cent distal small-bowel bypass; C, 33 per cent distal small bowel resection

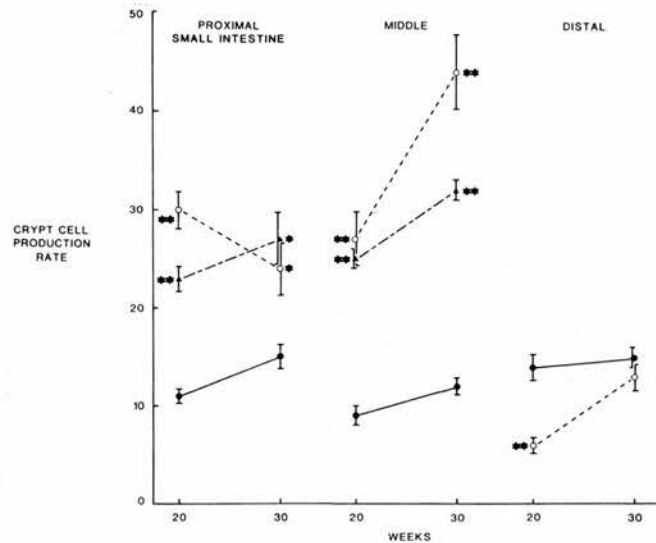


Figure 3 Crypt cell production rates (cells/crypt per hour \pm s.e.) in the small intestine 20 and 30 weeks after distal small-bowel bypass and distal small bowel resection. Significance: vs. controls: ** $P < 0.001$; * $P < 0.01$; ●, controls; ○, DSBB; ▲, DSBR

All rats dying before week 20 were excluded from the analysis. The nine rats that died spontaneously thereafter were included in the overall analysis of tumour yields but not in the results obtained from the individual planned sacrifices at 20, 25 and 30 weeks. Overall, 91 animals (67 per cent) survived until sacrifice (Figure 1), the mortality rate being slightly higher in the DSBR group (40 per cent) than in the DSBB (27 per cent) and control groups (31 per cent).

Body weight

Within a week of operation mean body weights exceeded pre-operative values to a similar degree in all three groups. From the tenth week onwards rats with DSBR gained weight more slowly than the others, and at the end of the experiment their mean weight (506 ± 15 g; \pm s.e.) was 10 per cent lower than the DSBB group (560 ± 12 g) and controls (568 ± 9) ($P < 0.01$).

Intestinal adaptation

No differences were found in the length and wet weight of the duodenum, colorectum or proximal two-thirds of the jejunum. After DSBB the excluded loop weighed only 66 per cent of the equivalent segment in controls. The wet weight of the caecum was 53–65 per cent higher after DSBR

(2.8 ± 0.1 g) and DSBB (2.6 ± 0.1 g) than in controls (1.7 ± 0.1 g; $P < 0.05$).

Crypt cell production rate increased with age (Figures 3 and 4). The overall CCPR of the small intestine was estimated by fitting a single regression line to the pooled data from the residual functioning segments in all groups. It accelerated from 14 to 24 cells/crypt per hour between 20–30 weeks, an increase of 71 per cent ($P < 0.01$). The overall CCPR of the large intestine was identical to that of the small bowel at 20 weeks and accelerated from 14 to 19 cells/crypt per hour by 30 weeks (35 per cent increase: $P < 0.01$).

At 20 weeks CCPRs in control large and small intestine varied between 8–15 cells/crypt per hour; rates after DSBB and DSBR were 109–200 per cent higher in the functioning small intestine (Figure 3) and up to 100 per cent higher in the large intestine (Figure 4). By 30 weeks the gap had generally widened, though not in the distal colon. Percentage increments were highest in the mid small bowel and proximal colon. CCPR in the caecum was slow to respond to DSBB and DSBR but at 30 weeks matched that of the proximal colon, with increments of 77 and 108 per cent over controls (Figure 4). There was no consistent difference in the degree of adaptive hyperplasia to DSBR and DSBB at either 20 weeks or 30 weeks. The lowest CCPR (6 cells/crypt per hour) was observed in the bypassed loop at 20 weeks and was less than half that in the equivalent control segment (14: $P < 0.01$) (Figure 3).

Intestinal tumours

All but 1 or 2 rats in each group developed at least one colorectal tumour. Tumour yields tended to increase with time after exposure to carcinogen in control rats and those with DSBB, though after DSBR yields were already near maximal at 20 weeks (Figure 5). The overall yield in the DSBR group (5.3 ± 0.5 tumours per rat; mean \pm s.e.) was 55 per cent higher than in controls (3.4 ± 0.5 ; $P < 0.02$). The overall yield in the DSBB group (4.5 ± 0.5) was 32 per cent higher than in controls, but this difference did not quite attain statistical significance.

At 20 weeks the DSBR tumour yield was twice that of controls ($P < 0.02$) and 58 per cent greater than after DSBB (Figure 5). By 25 weeks the difference between DSBR and DSBB yields had narrowed to 33 per cent; both operations produced more than twice as many tumours as controls. At 30 weeks, however, tumour yields were almost identical in the three groups.

At 20 weeks the mean tumour diameter in rats with DSBR (3.8 ± 0.3 mm) was 41 per cent greater than that of controls

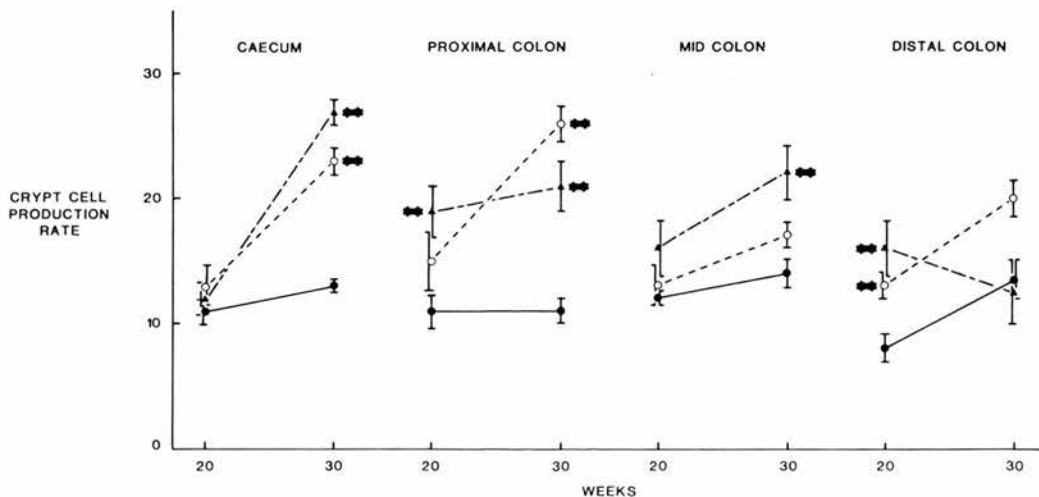


Figure 4 Crypt cell production rates (cells/crypt per hour \pm s.e.) in the large intestine 20 and 30 weeks after distal small-bowel bypass and distal small-bowel resection. Significance: vs. controls: ** $P < 0.01$; ●, controls; ○, DSBB; ▲, DSBR

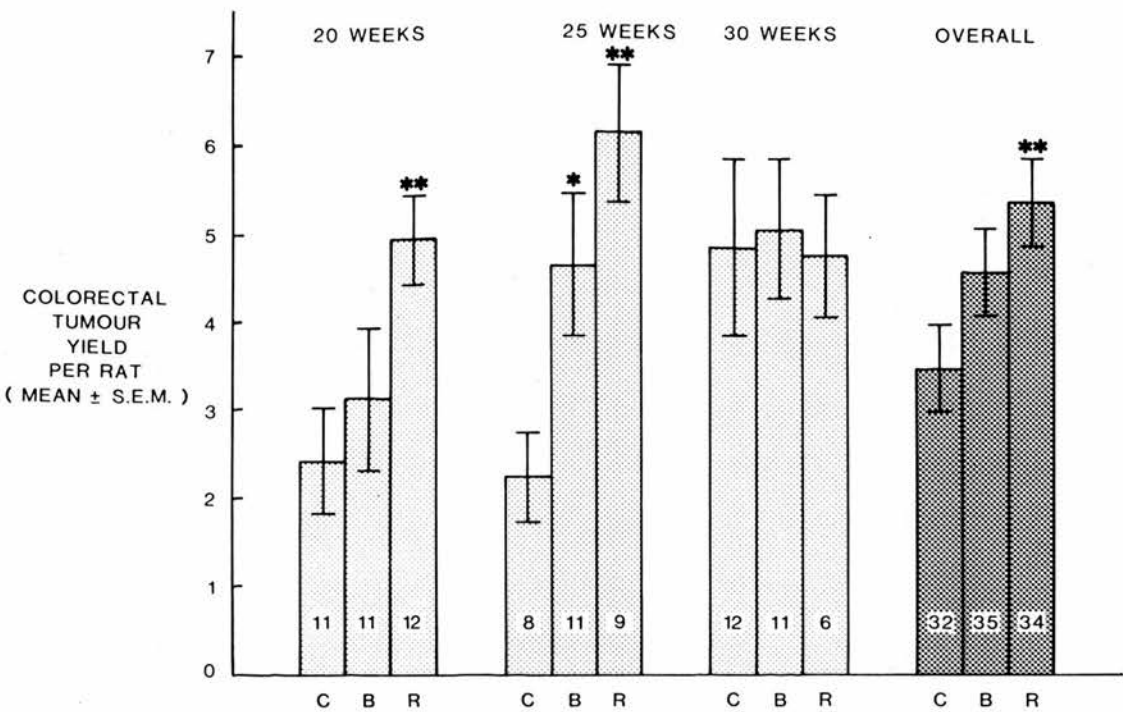


Figure 5 Colorectal tumour yields (mean \pm s.e.) both at staged sacrifices and overall. C = controls, B = distal small-bowel bypass, R = distal small-bowel resection. Numbers in each group are shown. Significance vs. controls: **, $P < 0.02$; *, $P < 0.05$

and 15 per cent greater than that of rats with DSBB (Figure 6). These differences had disappeared by 30 weeks. Compared to the 20 week measurements, mean tumour size had increased by 85 per cent in controls and by 70 per cent in the DSBB group ($P < 0.01$), but there was a much smaller increase (24 per cent) in the DSBR group. Overall there were no significant differences in tumour size between the three groups.

The pattern of colorectal tumour distribution was similar in the three groups (Figure 7): overall, 84 per cent of tumours developed in the distal 60 per cent of the large bowel. At 20 weeks the mean number of tumours found in the proximal 40 per cent was 1.2 ± 0.1 per rat in the DSBR group, 0.5 ± 0.2 in the DSBB group ($P < 0.01$) and 0.1 ± 0.1 in controls ($P < 0.02$). The yield of proximal tumours was similar in all groups at 30 weeks, however.

Tumours arose in the duodenum ($n=63$) and jejunum (15), but their incidence was not affected by operation or time of death. In addition four rats had caecal tumours, and twelve had tumours of the external auditory canal. Irrespective of operation, metastases were commoner after 25 weeks; usual sites included lymph nodes and omentum, liver, lungs and pleura. Carcinomatosis peritonei developed in four rats.

Adenocarcinoma was the commonest histological type (71 per cent), varying from carcinoma *in situ* to invasive cancer. Characterized by signet ring cells, mucinous (colloid) adenocarcinomas (5 per cent) mostly arose in the duodenum and were all deeply invasive. Benign adenomas and hyperplastic polyps accounted for the remainder of tumours. No differences in tumour histology were observed between the groups.

Discussion

This study confirms that distal enterectomy promotes experimental colorectal carcinogenesis and indicates that distal enteric bypass has a similar but less pronounced effect. Ileal resection has previously been shown to enhance carcinogenesis^{3,6,12}, but varying degrees of small bowel bypass have produced conflicting results^{4,7-9}. Scudamore and Freeman have recently found that proximal jejunoileal resection and bypass promote carcinogenesis to the same

extent¹³. The lesser effect of distal ileal bypass in promoting colorectal carcinogenesis cannot be explained by lower body weight. Although rats generally weigh less after bypass than after equivalent resection or transection⁴, no weight differentials were observed in the present experiment, perhaps because preservation of the ileocaecal valve prevented gross bacterial colonization of the blind loop. The lower body weight of rats with DSBR during the later stages of the experiment probably reflected their earlier development of intestinal cancers.

Both excision and exclusion of the ileum caused a substantial increase in intestinal crypt cell proliferation on either side of the line of resection. This compensatory hyperplasia occurred throughout the intestinal tract from the proximal jejunum to the distal colon, but changes were most marked in the mid small bowel and proximal large bowel. We have often observed this pattern of a generalized adaptive response, which is maximal adjacent to the

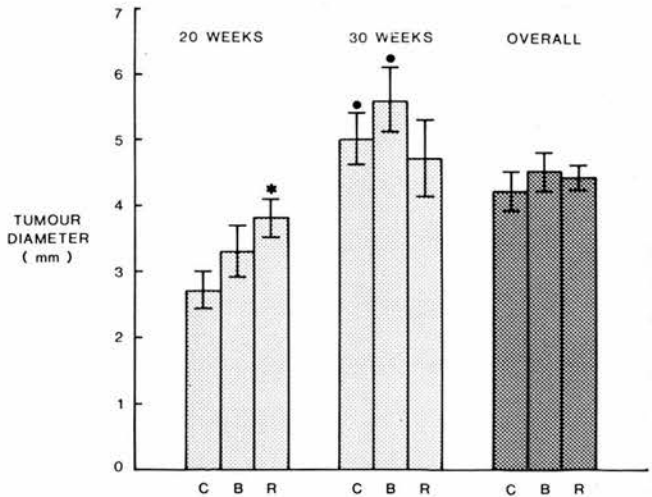


Figure 6 Colorectal tumour diameter (mm) (mean \pm s.e.) both at 20 weeks and 30 weeks and overall. C = controls, B = distal small-bowel bypass, R = distal small-bowel resection. Significance: *, $P < 0.02$ vs. controls; •, $P < 0.05$ vs. 20 week values.

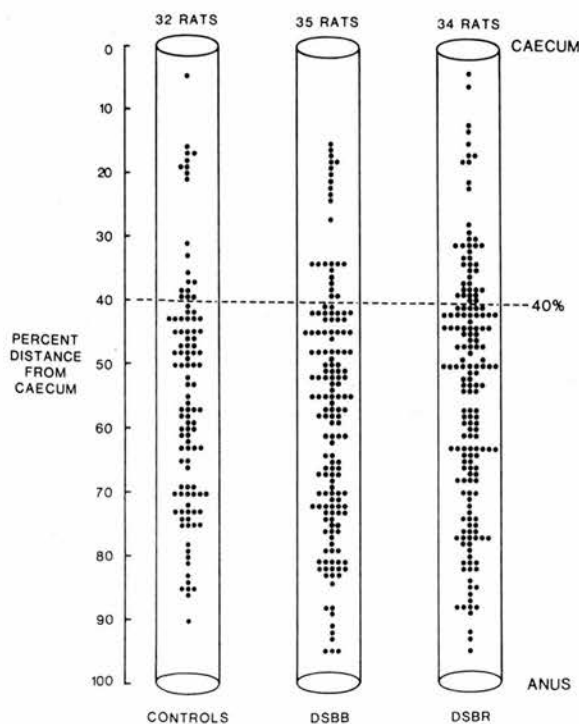


Figure 7 Colorectal tumour distribution after distal small-bowel bypass (DSBB) and resection (DSBR)

'missing' segment^{1-3,14}. After both resection and bypass intestinal growth continued between 20 and 30 weeks. It was presumably supplemented by the known hyperplastic effect of azoxymethane¹⁴. We have previously shown that the adaptive response to jejunal resection exceeds that observed after jejunal bypass during the first postoperative week, but by 4 weeks the discrepancy has vanished². Although adaptive changes in the small bowel persist for at least 3-6 months and probably indefinitely^{1,2,15}, the lesser degrees of colonic adaptation have proved more difficult to detect at this stage using measurements of wet weight or crypt depth^{4,12}. In the present study the normal wet weight of the large intestine belied the substantial increase in crypt cell production rate 20-30 weeks after ileal resection or bypass.

Initially (at 20 weeks) ileal resection produced greater numbers of tumours than controls or rats with ileal bypass. These tumours were also larger, and they developed particularly in the proximal colon, where the maximal adaptive effect was observed. By 25 weeks ileal bypass also demonstrated a significant cocarcinogenic effect, whereas at 30 weeks the yield, size and distribution of tumours were similar in all three groups, although only a small number of rats in the resection group had survived to this stage. These findings suggest that a major factor in the promotion of neoplasia by postoperative hyperplasia is a reduction in the latent period between exposure to the carcinogen and tumour development¹⁶. The first dose of carcinogen was given 1 week postoperatively, when the adaptive response to ilectomy would be clearly established, and the course was completed 5 weeks later, by which time the response to bypass would be expected to be of similar intensity.

Hyperplasia might predispose to neoplasia by increasing the number of intestinal epithelial cells exposed to the carcinogen¹⁷, or it could 'fix' any mutation by allowing rapid division of transformed cells and the establishment of a clone¹⁸. Creation and subsequent closure of a defunctioning transverse colostomy enhances carcinogenesis in the distal colorectum, even though the carcinogen is given immediately after colostomy closure, when the colon is still severely hypoplastic¹⁹. These data suggest that the number of proliferating cells is more important than the total number

of epithelial cells. Raised polyamine levels accompany cellular proliferation, and preventing the biosynthesis of polyamines by specific inhibition of ornithine decarboxylase lowers the incidence of colonic tumours induced by dimethylhydrazine²⁰.

Loss of functioning tissue and changes in food intake are the strongest stimuli to adaptive change in the intestinal tract, but the regulatory mechanisms involved are complex and interrelated^{1,21}. Luminal nutrients are of central importance, since parenteral nutrition curtails the compensatory response and leads to progressive atrophy of the gut²². Food probably has a direct trophic effect on intestinal epithelium besides provoking the release of alimentary hormones and secretions, which undoubtedly have a part to play²³. Enough evidence has now accrued to implicate enteroglucagon as a major enterotropic hormone²⁴. Nevertheless, our preliminary data suggest that the hyperplasia and increased neoplasia found in functioning colon after jejunoileal bypass⁸ are absent in colon defunctioned by a proximal colostomy, despite a three-fold increase in circulating enteroglucagon levels²⁵. Thus luminal factors seem to prevail over systemic factors in the control of intestinal adaptation and carcinogenesis.

If it were possible to extrapolate from small laboratory animals to man, our observations would have a disturbing relevance for the many young patients with Crohn's disease who undergo ileal resection and for those otherwise healthy patients in whom distal ileal bypass is carried out to control hyperlipidaemia.

Acknowledgements

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The cocarcinogenic effect of intrarectal deoxycholate in rats is reduced by oral metronidazole

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Summary Bile acids enhance colorectal carcinogenesis in animals and man, perhaps after degradation by faecal anaerobes. The promotional effect of sodium deoxycholate (SDC) and its relationship to bacteria was examined in male Sprague-Dawley rats ($n=115$) which had received a 6-week course of azoxymethane (total dose $90 \text{ mg kg}^{-1} \text{ s.c.}$). Two groups received $3 \times$ weekly intrarectal (i.r.) instillations of N saline or 0.12 M SDC for 18 weeks. Another group received SDC i.r. plus metronidazole (22.5 mg kg^{-1}) daily in the drinking water. Controls had no instillations or metronidazole alone. By 28 weeks SDC had increased mean colonic crypt depth by 9% ($P<0.001$), and had almost trebled colorectal tumour yields from 2.4 ± 0.4 per rat (mean \pm s.e.) in controls to 6.4 ± 0.5 ($P<0.001$). Tumour yields after SDC + metronidazole (4.2 ± 0.5) remained 75% higher than in controls ($P<0.01$) but were 33% less than after SDC alone ($P<0.01$), and the increase in crypt depth was maintained at 7% ($P<0.001$). Neither metronidazole alone nor saline i.r. had any effect on tumour yield, but metronidazole alone reduced crypt depth by 9% ($P<0.001$). Deoxycholate is a potent cocarcinogen and also stimulates mucosal hyperplasia. Metronidazole reduces its tumour-promoting effect, suggesting that faecal anaerobes are important in bile acid cocarcinogenesis.

Bile acids are strong candidates for the role of endogenous promoters of colorectal cancer (Reddy, 1981; Thompson, 1982). They bear a close steric resemblance to an established group of carcinogens, the polycyclic aromatic hydrocarbons. Human gut flora can cause partial aromatization of the steroid ring, and full conversion to 3-methylcholanthrene might be achieved by a series of chemical reactions (Hill, 1971). Faecal excretion of bile acids is greater in populations from high-risk countries (Western Europe, USA) as opposed to low-risk countries (Asia, Africa), and in meat eaters as opposed to vegetarians (Reddy & Wynder, 1973; Hill *et al.*, 1971; Aries *et al.*, 1969). Deoxycholic acid receptors have been identified in human colorectal cancers (Summerton *et al.*, 1982).

In theory, bile acids and their metabolites could either exert a direct mutagenic effect on the epithelial cell or act indirectly by altering the rate of mucosal cell proliferation and hence susceptibility to carcinogenesis. In the rat diversion of pancreaticobiliary secretions to mid small bowel enhances colorectal carcinogenesis (Chomchai *et al.*, 1974; Williamson *et al.*, 1979) and also stimulates marked adaptive hyperplasia of the ileum and moderate colonic hyperplasia (Williamson *et al.*, 1978). However, bile acids appear to be neither tropic nor cocarcinogenic to hypoplastic defunctioned colon (Rainey *et al.*, 1983). The composition of the colonic microbial flora is

implicated as the key intermediary modulating the effect of luminal bile acids (Aries *et al.*, 1969; Hill, 1979). Anaerobes, in particular, may metabolise bile acids to yield products which are carcinogenic or cocarcinogenic. (Hill, 1974).

This experiment was designed to test the tropic and cocarcinogenic potential of sodium deoxycholate instilled directly into the large bowel of rats exposed to azoxymethane. In addition we examined the effect of oral metronidazole, an anaerobicide, in modifying this potential.

Materials and methods

Experimental animals

One hundred and fifteen male Sprague-Dawley rats (Olac SD, Bicester, Oxon) weighing 70-100 g were received into the animal house 1 week before the start of the experiment and were allocated to one of five groups (Figure 1). They were fed standard rat chow (Oxoid Breeding Diet; H C Styles & Co Ltd., Bewdley, Worcs) and water *ad libitum*. Animal quarters were lit in alternate 12-hourly cycles. Rats were weighed weekly throughout the experiment. All animals received weekly s.c. injections of azoxymethane (Ash Stevens Inc., Detroit, Michigan, USA) 15 mg kg^{-1} for 6 weeks (Figure 1).

One week after the last injection of azoxymethane intrarectal (i.r.) instillations were carried out for the first time in groups 3-5. Colonic washout was not carried out and anaesthesia was unnecessary. An 18-gauge plastic i.v. cannula was

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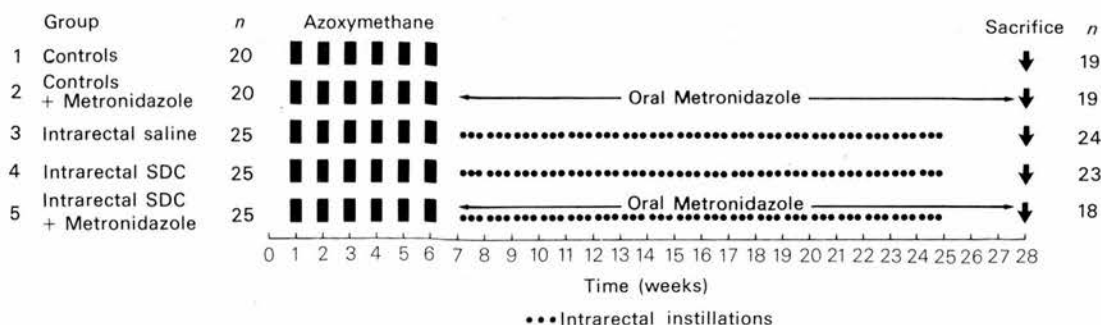


Figure 1 Experimental design. SDC=sodium deoxycholate. Numbers in each group at the start of the experiment and surviving until sacrifice are shown.

inserted through the anus to a distance of 5 cm in rats suitably restrained by an experienced handler. Rats in group 3 received 1 ml N saline. Groups 4 and 5 received 1 ml of 0.12 M sodium deoxycholate (SDC) prepared by dissolving 50 g SDC (Sigma Chemical Co., St Louis, USA) in 1 litre N saline; each 1 ml aliquot contained 0.05 g (120 μ mol) of SDC. Instillations were carried out 3 times per week for 18 weeks (Figure 1).

In addition, rats in group 5 received metronidazole (22.5 mg kg⁻¹ rat day⁻¹; May & Baker Ltd., Dagenham, Essex) dissolved in the drinking water from the start of the instillations until the end of the experiment. Group 1 rats (controls) received neither i.r. instillations nor metronidazole, and those in group 2 received metronidazole alone (Figure 1).

Autopsy specimens

Rats were regularly examined for evidence of tumour development and were killed when moribund or at the end of 28 weeks. At autopsy the entire intestinal tract was excised. The following segments were thoroughly flushed with cold saline to remove all content: duodenum, jejunum and ileum and colorectum. The length of each segment was determined by suspension with a constant weight against a ruler. Segments were then opened, and the number, size and position of all tumours were recorded. After excision of the tumours the remaining bowel was blotted dry and weighed. The net weights of the caecum, liver, kidneys and spleen were also recorded. All tumours were fixed in 10% formalin before histological processing. Subsequently 5 μ m sections were prepared for staining with haematoxylin and eosin.

In addition, a 1 cm specimen of colon was excised 5–6 cm from the anus, and similar histological specimens were prepared. The mean crypt depth was estimated by ocular micrometry of 10 perfectly-sectioned crypts per slide.

Statistics

Student's *t*-test was used for statistical analysis of the data.

Results

Mortality rate

Eleven rats (10%) died before sacrifice either from colonic perforation during instillation (2 rats), haemorrhage secondary to duodenal or colonic cancer (2), haematuria (1), pneumonia (2), or cancer of the external auditory canal (1). In 3 rats that died during the early part of the experiment, the cause of death could not be determined. The yields of surviving animals at the end of the experiment are given in Figure 1.

Body weight

Rats in all groups gained weight steadily until week 25, after which weights remained constant until sacrifice 3 weeks later. Neither i.r. deoxycholate nor oral metronidazole had any consistent effect on body wt.

Intestinal adaptation

No differences between the groups were found in the lengths and weights of the duodenum, jejunum or colorectum, nor in the weights of the caecum, liver, kidneys or spleen.

The mean colonic crypt depth in controls was $226 \pm 3 \mu$ m (mean \pm s.e.) compared with $246 \pm 3 \mu$ m in the SDC-irrigated group and $242 \pm 3 \mu$ m in the SDC+metronidazole group ($P < 0.001$; Figure 2). Intrarectal saline had no effect on crypt depth compared with controls, but metronidazole alone produced a 9% decrease ($211 \pm 3 \mu$ m; $P < 0.001$).

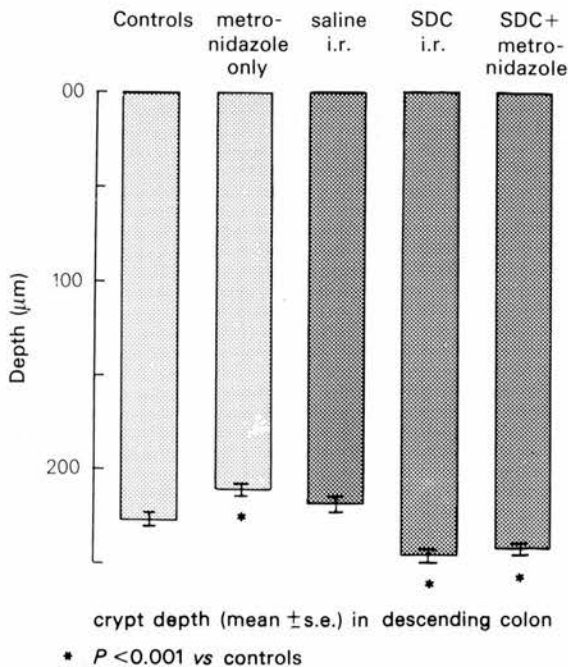


Figure 2 Colonic crypt depth at sacrifice (28 weeks). SDC=sodium deoxycholate.

Intestinal tumours

All but 2–3 rats in each group developed one or more colorectal tumours (Figure 3). Intrarectal deoxycholate almost trebled colorectal tumour yields from 2.4 ± 0.4 per rat (mean \pm s.e.) in controls to 6.4 ± 0.5 ($P < 0.001$). Metronidazole reduced this effect by 33% ($P < 0.01$), but the tumour yield (4.2 ± 0.5) remained 75% higher than that in controls ($P < 0.01$). Neither metronidazole alone (2.2 ± 0.6 tumours per rat) nor i.r. saline (2.8 ± 0.5) had any effect on colorectal carcinogenesis. No significant differences in tumour size were found between groups.

The overall pattern of colorectal tumour distribution was similar in the 5 groups. Ninety-six percent of all tumours developed in the distal 60% of the large bowel (Figure 4). The effect of SDC instillation was maximal in the distal 40% segment, where it produced a 193% increase in tumour yield over controls ($P < 0.01$), while the increase proximal to this was only 14%. Clearly the instillations were effectively reaching this distal 40% segment.

Tumours also arose in the duodenum ($n=5$) and jejunum (3), but their incidence was unaffected by SDC or metronidazole administration. In addition, 3 rats developed tumours of the external auditory canal, and metastases were found in the lung, liver and omentum.

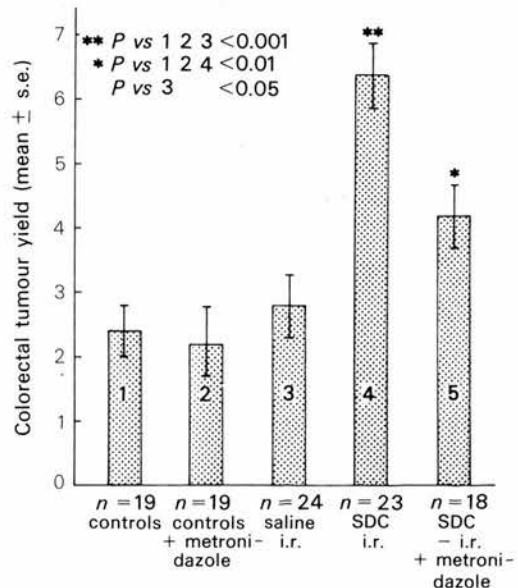


Figure 3 Colorectal tumour yields. SDC=sodium deoxycholate.

Histological types of benign and malignant neoplasms were as previously described (Williamson *et al.*, 1979; Rainey *et al.*, 1983).

Discussion

The data support the contention that sodium deoxycholate is a potent promoter of experimental colorectal carcinogenesis. Oral administration of primary bile acids has increased tumour yields in various models: rats and mice given dimethylhydrazine (Martin *et al.*, 1981) or methylnitrosourea (Cohen *et al.*, 1980), and rats with 'spontaneous' cancers arising at a colostomy (Sauer *et al.*, 1980). Direct exposure of colorectal mucosa to primary or secondary bile acid solutions instilled per rectum also promotes carcinogenesis in response to the contact carcinogen N-methyl-N'-nitro-N-nitroso-guanidine (Narisawa *et al.*, 1974; Reddy *et al.*, 1977). These experimental data are supported by a wealth of epidemiological surveys identifying bile acids as major cocarcinogens (Reddy, 1981; Thompson, 1982).

The mechanism of action of bile acids in colonic carcinogenesis has not been elucidated. They might directly damage the epithelial cell: lithocholic acid can induce DNA strand breaks in cultured cells (Kulkarni *et al.*, 1982). Bile acids are tropic to ileal mucosa (Williamson *et al.*, 1978), and our finding that SDC increases colonic crypt depth indicates

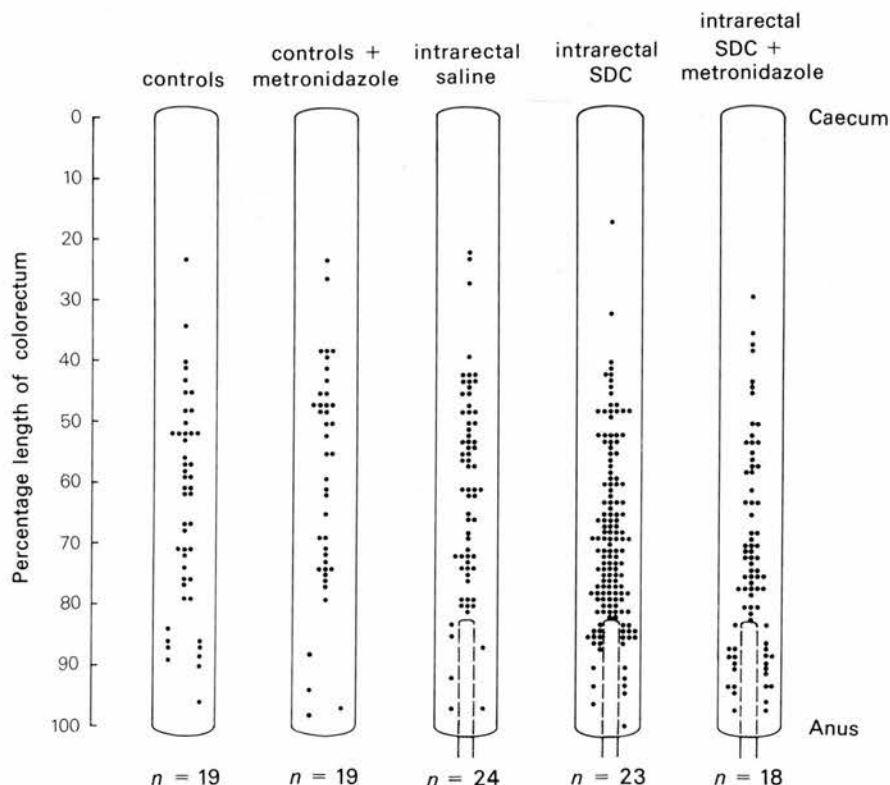


Figure 4 Colorectal tumour distribution. SDC=sodium deoxycholate. Estimated distance of i.r. cannula insertion is shown.

that they produce a similar response in colonic mucosa. Hyperplasia is a strong promoter of experimental intestinal cancer (Williamson 1982a; Barthold, 1981). Both varying degrees of small bowel resection and pancreaticobiliary diversion to mid small bowel result in moderate colonic hyperplasia and the enhancement of colorectal carcinogenesis (Williamson, 1982a). The tropic effects of SDC on colonic mucosa might therefore be sufficient to explain its tumour-promoting effect. Possibly bile acids produce hyperplasia by causing chronic irritation and inflammation of the mucosa, rendering it more susceptible to carcinogenesis. Certainly, the chronic inflammation of ulcerative colitis in man increases the risk of colorectal cancer (Lennard-Jones *et al.*, 1977; van Heerden & Beart, 1980). We have recently found that isolating a long segment of colon from the faecal stream as a Thiry-Vella fistula produces both mucosal hypoplasia and reduced susceptibility to azoxymethane (Rainey *et al.*, 1983). SDC instillation into this defunctioned colon has no effect on the reduced tumour yield or the mucosal hypoplasia. Clearly SDC requires the presence of faeces or some faecal constituent in

order to exert its cocarcinogenic effects. Absent in defunctioned bowel, the mechanical stimulus of faecal bulk may be important in maintaining normal mucosal cell turnover (Williamson, 1982b). Similarly a normal bacterial flora is necessary for maintenance of the normal mucosal proliferative state (Abrams *et al.*, 1962), and its composition may modulate carcinogenesis (Hill, 1979). The bacterial population in a defunctioned Thiry-Vella fistula is probably very different both qualitatively and quantitatively from that in normal functioning colon.

In this study, metronidazole had no effect on carcinogenesis in response to azoxymethane alone. Yet Goldin & Gorbach (1981) have found that the administration of tetracycline or erythromycin to rats receiving dimethylhydrazine markedly reduces colorectal carcinogenesis; these antibiotics have a different spectrum of antibacterial activity than metronidazole. Since chemical carcinogenesis is also reduced in germ-free rats (Reddy *et al.*, 1975a), it is possible that the dose of metronidazole did not reduce the population and metabolic activity of colonic bacteria enough to inhibit carcinogenesis.

Nevertheless there was a slight but significant reduction in colonic crypt depth, similar to that found in the ileum of germ-free mice (Abrams *et al.*, 1962).

The importance of faecal anaerobes in the cocarcinogenic role of bile acids is highlighted by the finding that metronidazole partly suppresses the effect of intrarectal SDC. Nuclear-dehydrogenerating clostridia in particular are capable of producing unsaturated steroids from the bile acid nucleus (Hill, 1974). These organisms may be more numerous in the faeces of patients with colorectal cancer than in control groups (Hill, 1975; Murray *et al.*, 1980). In man, high fat/low protein diets increase the total anaerobic microfloral content of the faeces as well as the activity of the bacterial enzyme beta-glucuronidase (Reddy & Wynder, 1973; Reddy *et al.*, 1975b; Goldin & Gorbach, 1976). The concentration of faecal anaerobes in one high-risk population (British) exceeded that of a low-risk population (Ugandan) especially for those

bacteria capable of degrading bile acids (Hill *et al.*, 1971; Aries *et al.*, 1969). Other studies have found no difference in bacterial populations between groups at varying risk (Moore & Holdeman, 1975; Finegold *et al.*, 1975), so that the metabolic activity of the microbial flora may be more relevant than the actual numbers of individual species (Reddy *et al.*, 1980). Since bile acids remain cocarcinogenic in germ-free rats, the presence of bacteria is clearly not essential (Reddy *et al.*, 1977). Similarly in this experiment, although metronidazole reduced the promotional effect of intrarectal SDC, deoxycholate remained strongly cocarcinogenic.

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